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Title of Thesis: "The Effects of Nicotine on MK-801-induced
Attentional Deficits: An Animal Model of
Schizophrenia"

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ABSTRACT

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Attentional Deficits: An Animal Model of

Schizophrenia

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The present study consisted of three experiments. Experiment 1 examined the effects of acute nicotine (0, 0.1 or 0.5 mg/kg) on the acoustic startle reflex (ASR) and pre-pulse inhibition (PPI) in animals treated with MK-801, an NMDA antagonist that models schizophrenic attentional deficits. Experiment 2 examined the effects of acute nicotine (0, 0.01 or 1.0 mg/kg) on ASR and PPI in MK-801-treated animals. Experiment 3 examined the effects of chronic nicotine (0 or 6.0 mg/kg/day) on ASR and PPI in animals treated with MK-801. MK-801 consistently disrupted attention by increasing ASR and decreasing PPI in all experiments. Nicotine failed to consistently attenuate these attentional disruptions. Nicotine had no significant effects on ASR or PPI at any dosage and attenuated the decrease in PPI caused by MK-801 only in a single set of startle parameters. These findings suggest that this model of attentional deficits in schizophrenia is not affected by nicotine.

The Effects of Nicotine on MK-801-induced Attentional Deficits:

An Animal Model of Schizophrenia

by

Jennifer M. Phillips

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INTRODUCTION

Overview of Schizophrenia

Schizophrenia is a mental health disorder that affects approximately 2.2 million adults in the United States each year (Narrow, 1998). There are roughly 300,000 acute schizophrenic episodes every year in the United States (Stahl, 2000). Prevalence of schizophrenia is estimated to be approximately 1.0-1.3%, based upon data from various studies including the Epidemiological Catchment Area study (ECA) and the National Comorbidity Study (NCS) (USDHHS, 1999; APA, 2000). Support for a gender bias in schizophrenia is mixed with hospital-based studies suggesting a higher rate of the disorder in men, but community-based studies reporting approximately equal rates of the disorder across the genders. Schizophrenia tends to have a later onset and a better prognosis in women (Hafner et al., 1998; APA, 2000; Leung & Chue, 2000).

The cause of schizophrenia is not known although many theories have been proposed to explain this disorder. Immediate biological relatives of people with schizophrenia are approximately 10 times more likely to develop the disorder than members of the general population, suggesting a strong influence of genetics; substantial differences in disorder development rates among identical twins, though, emphasizes the importance of environmental factors (USDHHS, 1999; APA 2000). This has led researchers to propose that schizophrenia is caused by a genetic susceptibility in conjunction with outside stressors, the diathesis-stress model (Zubin & Spring, 1977). This model is currently the most well-accepted explanation for schizophrenia (USDHHS, 1999).

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According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR), the essential features and symptoms of schizophrenia can be divided into two categories: positive (symptoms that are present) and negative (the absence of normal behaviors) (APA, 2000). Positive symptoms are generally characterized by an exaggeration or distortion of normal functions and include delusions, hallucinations, disorganized speech, and catatonic behavior. The negative symptoms of schizophrenia are characterized by a decrease or loss of normal functioning. Examples of negative symptoms include flat affect, alogia - a restriction in the productivity of thought and speech, and avolition - a lack of initiation and goal-directed behavior. In addition, schizophrenics are often characterized by deficits in attention and information processing (Braff & Geyer, 1990; Braff, Swerdlow, & Geyer, 1995; Heinrichs & Zakzanis, 1998).

Treatments for Schizophrenia

Although there is no cure for schizophrenia, several types of treatments, including pharmacologic and psychosocial options, are available.   Medication is a common form of treatment for schizophrenics and is often relied upon to control the thought disorders, hallucinations, and delusions associated with the disorder. Medications can be used to decrease the symptomatology of schizophrenia to allow for the application of other forms of treatment, such as a psychosocial intervention.

The pharmacological treatment of schizophrenia is based upon a serendipitous finding from the 1950s. An antihistamine, chlorpromazine, was

observed to have antipsychotic effects when used in schizophrenic patients. Chlorpromazine (Thorazine) is a "conventional antipsychotic," or a neuroleptic - a class of drugs that also includes Haloperidol (Haldol) (Dixon, Lehman, & Levine, 1995; Love, 1996; Stahl, 2000). Conventional antipsychotics are rarely prescribed now because of their negative side effects including: cognitive and neurologic deficits (such as sedation, confusion, and tardive dyskinesia), gastrointestinal, cardiovascular, and endocrinological side effects (Gerlach, 1999; Bezchlibynyk-Butler & Jeffries, 2000; Kane, 2001).

The "novel antipsychotics," such as Risperidone (Risperdal), Clozapine (Clozaril), and Olanzapine (Zyprexa), were introduced in the mid 1990s and are more commonly prescribed today. There are still significant problems with cognitive and neurologic function in these patients although they are not as severe as those associated with the conventional antipsychotics (Gerlach, 1999; Bezchlibynyk-Butler & Jeffries, 2000; Kane, 2001).

Pharmacotherapy improves many of the symptoms of schizophrenia, but it does so at the price of significant negative side effects and has limited effects on the social impairments that characterize the disorder and limit normal functioning in those afflicted (Huxley, Rendall, & Sederer, 2000). Psychosocial therapy has been more successful in easing these impairments and facilitating normal social functioning in schizophrenic patients. Cognitive behavioral therapy (CBT), family and group therapy, as well as psychoanalysis have positive effects and enhance functioning (Huxley, Rendall, & Sederer, 2000; Marder, 2000; Rector & Beck, 2001). However, pharmacological interventions, and their accompanying side

effects, are often necessary to control the thought-disordered symptoms of the patients before these psychosocial interventions can be effective.

Although both pharmacotherapy and psychosocial therapies alleviate some of the symptoms of schizophrenia, there are still aspects of the disorder that remain resistant to treatment. Treatment to date has relied upon a process of symptom management rather than a cure for the disorder. In consideration of these deficits, as well as the detrimental side effects of certain existing treatments, continued research into alternative effective, low-risk treatments is necessary.

Schizophrenia and Cigarette Smoking

The tendency for individuals suffering from mental health disorders to abuse drugs has frequently been reported (Helzer & Pryzbeck, 1988; Regier et al., 1993; Batel, 2000). The Epidemiologic Catchment Area Study (ECAS) estimated lifetime rates of substance abuse and addictions to range from 32 to 56% in those suffering from major psychiatric disorders such as Bipolar Disorder, Major Depressive Disorder, and Schizophrenia (Regier et al., 1993). The estimate of lifetime prevalence of illicit drug use in the general population is approximately 38% (Substance Abuse and Mental Health Services Association, 2000).

Schizophrenics, in particular, abuse drugs and alcohol (Dixon, Haas, Weiden, Sweeney, & Frances, 1991; Batel, 2000). One commonly used drug in schizophrenic patients is tobacco, predominantly in the form of cigarette

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smoking. Although various studies note different prevalence rates for smoking in

schizophrenia, they are all substantially higher than the prevalence of smoking in

the general population which has remained at approximately 24% over the past

decade (CDC, 2001). In a study of 277 psychiatric outpatients compared with

local and national population based samples, schizophrenics were found to have

a smoking rate of 88%. The control population smoked at a rate of

approximately 30%, much closer to the national average (Hughes, Hatuskami,

Mitchell, & Dahlgren, 1986).   A study of 360 inpatient psychiatric patients, male

and female, revealed that schizophrenic patients smoked at a rate of 88%,

significantly greater than those with non-schizophrenic diagnoses (67%) (de

Leon, Dadvand, Canuso, White, Stanilla, & Simpson, 1995). Additional inpatient

and outpatient studies have further supported this high prevalence of smoking in

schizophrenics, finding prevalence rates between 68 and 78% (Goff, Henderson,

& Amico, 1992; Ziedonis, Kosten, Glazer, & Frances, 1994).

Several explanations have been proposed for the greater prevalence of

cigarette smoking in schizophrenics. One hypothesis postulates that there may

be a biologic or genetic predisposition in certain individuals that makes them

susceptible to schizophrenia and drug addictions (Batel, 2000). The dopamine-

opioid neurotransmission system has been implicated in both schizophrenia and

in addiction and may explain the high prevalence of their co-occurrence (Batel,

2000).

A second hypothesis for the increased rates of cigarette smoking in

schizophrenics is that schizophrenics may use cigarette smoking to modulate the

symptoms of the disorder as well as possible side effects of the drugs used in its treatment (Goff, Henderson, & Amico, 1992; Dalack, Healy, & Meader-Woodruff, 1998; Batel, 2000). This hypothesis is known as the self-medication hypothesis. It has been proposed that smoking may decrease the anxiety, tension, and dysphoria common in schizophrenia (Batel, 2000) in addition to regulating affective deficits (Blanchard, Squires, Henry, Horan, Bogenschutz, Lauriello, et al., 1999). There is some evidence that smoking may facilitate social integration and relationships (Smith, 1996), alleviate medication side effects, particularly extrapyramidal side effects (Goff, Henderson, & Amico, 1992; Batel, 2000), and attenuate cognitive and attentional impairments (Newhouse & Hughes, 1991) in schizophrenics.

Cigarette Smoking

Approximately 24% of the American population currently smokes (CDC, 2001). Smoking has been linked to cardiovascular diseases, respiratory diseases, and various cancers (CDC, 1997). Smokers also are more likely to die from their problems. Smokers are three times as likely as non-smokers to die from coronary heart disease and are more than 10 times as likely to die from lung cancer (CDC, 1993). It is estimated that between 1990 and 1994, 2.1 million people in the United States died as a result of smoking-related health problems (CDC, 1997). Despite these grim statistics and the well-publicized health risks associated with smoking, the prevalence of smoking and smoking initiation has not changed dramatically in the past 20 years.

The principal explanation for continued smoking behavior is the reinforcing nature of nicotine use. Nicotine, the primary psychoactive ingredient in tobacco products and tobacco smoke, is recognized as a highly addictive substance (USDHHS, 1988). The psychopharmacologic effects of nicotine are highly reinforcing and diverse and act to relieve the unpleasant withdrawal symptoms associated with nicotine abstinence in regular smokers (Rose, 1996). Addiction to nicotine is believed to be the primary cause of persistent smoking behavior, despite adverse health effects.

While nicotine addiction provides a substantive argument for the maintenance of smoking behavior, there are several additional reasons for initiation and maintenance of this behavior. Social aspects of smoking appear to play a large role in these processes as well. Modeling of peers and family members consistently predicts cigarette use, especially in adolescent populations (USDHHS, 1994). Additional social pressures to smoke include perception of other smokers, the perceived instrumental value of smoking, and a risk-taking or rebellious nature (Camp, Klesges, & Relyea, 1993). Smokers often cite a reduction in stress and anxiety as a major reason for smoking (Frith, 1971; Spielberger, 1986; USDHHS, 1988). Finally, externally motivating factors, such as the tendency for smoking to decrease body weight, may also play a role in the decision to initiate and maintain cigarette smoking (Grunberg, 1990; Camp, Klesges, & Relyea, 1993).

In addition to the biological and social mechanisms that contribute to cigarette smoking, the effects of smoking on cognitive and attentional abilities

may contribute to maintenance of this behavior. Nicotine, delivered via cigarette

smoking, enhances various cognitive processes, including attention. Nicotine-

addicted smokers, when deprived of nicotine, experience impaired attentional

and cognitive abilities that can be restored by reinstatement of the drug

(Heishman, 1999).

In smokers and non-smokers, administration of nicotine is reported to

enhance attention. Nicotine, administered subcutaneously or through cigarette

smoking, has been reported to improve speed and accuracy in both smokers and

non-smokers on the rapid visual information processing test (Wesnes &

Warburton, 1983; Wesnes & Warburton, 1984; Foulds, Stapleton, Swettenham,

Bell, McSorley, & Russel, 1996). Nicotine also reportedly improved response

speed on the Stroop task in abstinent smokers and never smokers (Perkins,

Grobe, Epstein, Caggiula, Stiller, & Jacob, 1993).

Acoustic Startle Reflex and Pre-pulse Inhibition

The acoustic startle reflex (ASR) and pre-pulse inhibition of this reflex

(PPI) are believed to be behavioral responses that can be used to index cognitive

processes such as information processing and attention (Acri, Grunberg, &

Morse, 1991; Acri, 1994; Acri, Morse, Popke, & Grunberg, 1994; Acri, Brown,

Saah, & Grunberg, 1995; Faraday, O'Donoghue, & Grunberg, 1999a; Faraday,

2000; Swerdlow, Braff, Geyer, & Koob, 1986). The acoustic startle response is a

reflexive response present in all mammals that is characterized by an involuntary

series of muscular responses to a sudden, intense acoustic stimulus (Davis,

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1984). The ASR can be obtained using the same stimuli across species, including humans and rats (Swerdlow, Braff, Taaid, & Geyer, 1994). This paradigm has good face validity and generalizes from an animal model to human responses (Swerdlow, Caine, Braff, Taaid, & Geyer, 1992).

Pre-pulse inhibition (PPI) of the acoustic startle response (ASR) is achieved by presenting a non-startling acoustic stimulus shortly before the startling stimulus. A non-startling pre-pulse presented in such a manner results in measurably reduced startle responses (Braff, Stone, Callaway, Geyer, Glick, & Bali, 1978). Consistent with the acoustic startle reflex, PPI occurs in rats and humans. PPI is believed to measure sensorimotor gating, a mechanism by which an organism is able to select relevant stimuli from the environment while ignoring irrelevant information (Swerdlow, Caine, Braff, & Geyer, 1992).

Nicotine affects both of these behavioral responses. Pre-treatment with nicotine enhances ASR amplitude and increases PPI in male and female rats (Acri, 1994; Acri et al., 1994; Popke, 1996). These findings are consistent with reports that nicotine may enhance attention in smokers and non-smokers in many differing tasks, including ASR and PPI. Subcutaneous nicotine administered to healthy male non-smokers increased PPI in a dose-reponse manner (Kumari, Cotter, Checkley, & Gray, 1997). PPI was reportedly reduced in healthy, male overnight smoking-deprived smokers; cigarette smoking after deprivation restored PPI to pre-deprivation levels (Kumari, Checkley, & Gray, 1996). Smoking also reportedly enhances PPI in healthy male and female non-smokers, deprived smokers, and smoking-smokers in a gender dependent

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manner. Ad libitum smoking enhanced PPI in male smokers relative to male

non-smokers. In females, deprivation reduced PPI and smoking restored it

(Della Casa, Hofer, Weiner, & Feldon, 1998).

Deficits in ASR and PPI also have been used to measure cognitive and

attentional problems associated with various mental disorders. Schizophrenics in

particular are often characterized by deficits in attention and information

processing (Braff & Geyer, 1990; Braff, Swerdlow, & Geyer, 1995; Heinrichs &

Zakzanis, 1998).   Studies of schizophrenic patients report a significant deficit in

PPI that appears to support the presence of impaired sensorimotor gating in this

population (Geyer & Braff, 1987; Braff & Geyer, 1990; Braff, Grillon, & Geyer,

1992). PPI deficits are also consistent with self-reported attentional difficulties in

schizophrenics.

Animal Models

Animal models are used frequently in research to approximate human

conditions and allow for experimental manipulations and control that would be

impossible in clinical research.   They have been used to model a variety of

mental health conditions, including depression and anxiety (Treit, 1985; Palanza,

2001) schizophrenia, and substance abuse. The research that is pertinent to the

present studies includes animal models of tobacco use and schizophrenia.

Tobacco abuse: Models of tobacco and nicotine abuse utilizing multiple

species, particularly rodents, have made significant contributions to this area of

research. Self-administration of nicotine in rodents has been used to explore

factors such as predisposition to nicotine dependence and to develop treatment strategies (Rose & Corrigall, 1997; Corrigall, 2001). A chronic model of prolonged nicotine administration in rodents is reported to have effects on multiple behaviors including analgesia (Carstens, Anderson, Simons, Carstens, Mirela, & Jinks, 2001), activity and exploration (Faraday, Scheufele, Rahman, & Grunberg, 1999), social interaction (Scheufele, Faraday, & Grunberg, 2000), attention (Acri, Brown, Saah, & Grunberg, 1995), and body weight and food consumption (Grunberg, Bowen, Maycock, & Nespor, 1985), among others.   An acute model of nicotine administration, daily injections, is reported to have effects on several behavioral indices as well, including attention as measured by PPI (Acri, Morse, Popke, & Grunberg, 1994), as well as operant tasks and cognitive processing (Popke, Mayorga, Fogle, & Paula, 2000).

Schizophrenia: Schizophrenia also has been modeled in animals through various paradigms. These models are commonly not intended to model the entirety of the disorder in an animal. A general animal model of schizophrenia would be extremely difficult to produce given the strong emphasis on thought-disordered and self-report symptoms. They are more often designed to examine a particular deficit or symptom associated with the disorder, or a potential mechanism for its development (Marcotte, Pearson, & Srivastava, 2001). Models of schizophrenia are frequently used to test the ability of certain drugs to attenuate related symptoms. The testing of antipsychotics in animal models also is valuable to confirm the validity of the models themselves.

Past animal models of schizophrenia have used a variety of procedures to mimic various aspects of the disease. Manipulation of the social environment (Weiss & Feldon, 2001) and direct lesioning of the brain (Marcotte et al., 2001) are examples of models reported to replicate certain aspects of schizophrenia. The newest models tend to mimic the biological phenomenon of the disease, particularly those associated with cortical developmental changes, genetic characteristics, and abnormalities of glutamate transmission (Lipska & Weinberger, 2000).

Manipulations of the acoustic startle reflex (ASR) and pre-pulse inhibition (PPI) paradigms have been used specifically to model the attentional difficulties in schizophrenia (Swerdlow & Geyer, 1998; Weiss & Feldon, 2001). These measures in rodents have been demonstrated to be a valid model of human responses to similar stimuli in normal and schizophrenic individuals (Braff & Geyer, 1990).

MK-801 Model: The MK-801 model of the attentional deficits in schizophrenia combines two areas of schizophrenic modeling. MK-801, or dizocilpine, is an NMDA antagonist related in structure and actions to phencyclidine and ketamine. The use of this drug to model schizophrenic symptoms is based upon the glutamate hypothesis of schizophrenia. This hypothesis proposes that a glutamatergic hypofunction is an underlying cause of schizophrenia. MK-801 and related drugs have been reported to produce psychotic-like effects in humans (Lahti, Koffel, LaPorte, & Tamminga, 1995) and abnormal behaviors in rodents (Willets, Balster, & Leander, 1990) consistent with

previous animal models of schizophrenia. For example, MK-801 has been reported consistently to produce increases in the acoustic startle reflex and decreases in pre-pulse inhibition consistent with those observed in schizophrenic patients (Bakshi, Swerdlow, & Geyer, 1994; Varty & Higgins, 1995; Feifel, Reza, Wustro, & Davis, 1999; Varty, Bakshi, & Geyer, 1999).   These attentional deficits, in addition to other schizophrenic-like behaviors induced by MK-801 administration in rodents, may be antagonized by various typical and atypical antipsychotic medications (Arnt & Skarsfeldt, 1998; Smith, Boyer-Miller, & Goudie, 1999; Varty, Bakshi, & Geyer, 1999). These results lend further support to the use of MK-801, and the resulting attentional deficits, as a valid animal model of the attentional disruptions of schizophrenia.

Nicotine and the MK-801 Model: Other pharmacological agents may be effective to attenuate certain behaviors resulting from MK-801 administration. NIH Swiss mice exhibit discrete episodes of explosive jumping behavior described as "popping" following MK-801administration. This behavior has been proposed as a potential pre-clinical paradigm for the screening of novel antipsychotic medications. Both nicotine and mecamylamine, a nicotinic antagonist, reportedly block this "popping" behavior (Tizabi, Mastrapaolo, Park, Riggs, Powell, Rosse, & Deutsch, 1998). The ability of nicotine to block the occurrence of this behavior suggests that it may be of therapeutic benefit in the treatment of schizophrenia and psychotic behaviors, consistent with the self-medication hypothesis of tobacco use by schizophrenics.

The present experiments were conducted to examine the effects of pre-treatment with nicotine on sensorimotor and attentional deficits in the MK-801 animal model of the attentional deficits associated with schizophrenia. Experiment 1 examined the effects of nicotine administered acutely (0, 0.1 or 0.5 mg/kg) on the acoustic startle reflex (ASR) and pre-pulse inhibition (PPI) in animals treated with MK-801, an NMDA antagonist used to model attentional deficits associated with schizophrenia. Experiment 2 examined the effects of nicotine administered acutely (0, 0.01 or 1.0 mg/kg) on ASR and PPI in animals treated with MK-801. Experiment 3 examined the effects of nicotine administered chronically (0 or 6.0 mg/kg/day) on ASR and PPI in animals treated with MK-801.

It was hypothesized that nicotine administration would attenuate the enhancement of ASR and reverse deficits in PPI caused by treatment with MK-801. The hypothesized results would indicate a potential role of nicotine to alleviate some of the cognitive difficulties experienced by schizophrenics and would support the self-medication hypothesis.

EXPERIMENT 1

<u>Overview</u>

The purpose of Experiment 1 was to examine effects of acutely administered nicotine on the acoustic startle response (ASR) amplitude and pre-pulse inhibition (PPI) of acoustic startle in animals treated with MK-801, an NMDA receptor antagonist in a 2 (0 or 0.15 mg/kg MK-801) x 3 (0, 0.1, or 0.5 mg/kg nicotine) factorial design. Previous studies of animals treated with MK-801 have reported increased ASR amplitude and decreased PPI, suggesting that MK-801 may interrupt attentional processes and cause deficits in sensorimotor gating (Bakshi, Swerdlow, & Geyer, 1994; Varty & Higgins, 1995; Feifel, Reza, Wustro, & Davis, 1999; Varty, Bakshi, & Geyer, 1999). Based on previous studies of the effects of acute nicotine on ASR and PPI (Acri, Morse, Popke, & Grunberg, 1994; Popke, 1996), it was hypothesized that administration of 0.1 mg/kg or 0.5 mg/kg acute nicotine would counter these MK-801-induced deficits.

Following acclimation and baseline exposures to the acoustic startle equipment, animals were assigned to experimental conditions. On the day of testing, all animals received injections of nicotine (0, 0.1, or 0.5 mg/kg) and MK-801 (0 or 0.15 mg/kg) before being exposed to the startle and pre-pulse stimuli. Trials composed of a startle stimulus, 110 dB or 120 dB, with and without pre-pulse stimuli, 68 dB or 82 dB, were presented in a random order. ASR amplitude was measured using an interfaced computer while PPI was determined mathematically using ASR data from trials with and without pre-pulse stimuli.

Hypotheses

Hypothesis 1: It was hypothesized that MK-801 administration would significantly increase ASR amplitude.

Rationale: Several studies have reported a significant increase in the acoustic startle reflex of rats following administration of MK-801 (0.1 – 0.3 mg/kg) (Bakshi et al., 1994; Varty & Higgins, 1995; Feifel et al., 1999; Varty et al., 1999).

Hypothesis 2: It was hypothesized that MK-801 administration would significantly decrease percent PPI.

Rationale: A significant decrease in PPI has been reported following administration of MK-801 (0.1 – 0.3 mg/kg) in multiple studies (e.g. Bakshi et al., 1994; Varty & Higgins, 1995; Feifel et al., 1999; Varty et al., 1999).

Hypothesis 3: It was hypothesized that nicotine would significantly decrease the ASR amplitude.

Rationale: Acri et al. (1994) reported a decrease in the acoustic startle reflex of animals treated with acute doses of nicotine (0.5 mg/kg and higher).

Hypothesis 4: It was hypothesized that nicotine would significantly increase percent PPI.

Rationale: Acri et al. (1994) reported significant increases in percent PPI in animals treated with acute nicotine (0.001 and 0.01 mg/kg). In addition, Popke

(1996) reported that animals treated acutely with 0.5 mg/kg nicotine had greater PPI than did saline controls.

Hypothesis 5: It was hypothesized that there would be a significant Nicotine X MK-801 interaction for percent PPI.

Rationale: Based upon previous findings that nicotine increases PPI while MK-801 decreases it, it was hypothesized that an interaction would result such that animals receiving both nicotine and MK-801 would demonstrate greater PPI than those receiving MK-801 alone. Nicotine was hypothesized to effectively minimize or reverse the effects of MK-801 on PPI.

Methods

Subjects

Subjects were 61 adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA). Male subjects were used for several reasons including to prevent an unmanageable study size and to avoid hormonal variation related to the estrus cycle in females. In addition, hospital-based studies suggest that schizophrenia is more prevalent in males than females (APA, 2000). There were 10 animals per cell (11 animals in the MK-801/saline group). This cell size was based upon previous research that reported significant effects of acute nicotine (Acri, 1992; Acri et al., 1994; Popke, 1996) and MK-801 (Al-Amin & Schwarkopf, 1996; Varty, Bakshi, & Geyer, 1999) on ASR and PPI. Animals were aged 50-52 days and weighed 225-250 g at the beginning of the

experiment. All subjects were pair-housed in standard polypropylene rectangular cages (35.6 cm x 15.2 cm x 20.3 cm) on hardwood chip bedding (Pine-Dri) with continual access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained on a 12-hour reverse light/dark cycle (lights on at 1730 hours) at a temperature of 23°C and 50 percent relative humidity.

Equipment

The acoustic startle reflex (ASR) and pre-pulse inhibition (PPI) were measured using the Med Associates Acoustic Response Test System (Med Associates, Georgia, VT). The equipment consisted of eight weight-sensitive platforms located inside separate sound-attenuated and ventilated chambers. Subjects were placed in cylindrical Plexiglas holders that were then affixed to the weight-sensitive platforms. The holders were sized to allow animals room to turn around and make other small movements, but to restrict large movements and extensive locomotion. The movements and weight displacement of subjects in response to stimuli were measured and recorded by an interfaced computer. Responses were recorded as the maximum response or weight displacement within 200 ms following the onset of the startle-eliciting stimulus.

Subjects experienced a 3-minute adaptation period following placement of the holders into the individual chambers during which no startle stimuli or pre-pulses were presented. Startle stimuli were white noise bursts of either 110 or 120 dB with a duration of 20 ms. These startle stimuli were sometimes preceded by 100 ms by pre-pulses of 68 or 82 dB, 1kHz pure tones. A low and high startle

and pre-pulse stimuli were chosen in an attempt to capture potential drug effects over a range of stimuli. These startle and pre-pulse stimuli values were chosen based on literature reporting significant effects of nicotine (Faraday et al., 1999a; 1999b) and MK-801 (Bakshi, Swerdlow, & Geyer, 1994; Varty & Higgins, 1995; Feifel et al., 1999; Varty et al., 1999) on ASR and PPI at stimuli of these magnitudes. Startle and pre-pulse stimuli decibel levels were verified by a Larson-Davis Sound Pressure Machine Model 2800 (Provo, Utah). There were six trial types: 1) 110 dB stimulus alone, 2) 120 dB stimulus alone, 3) 110 dB stimulus preceded by 68 dB pre-pulse, 4) 120 dB stimulus preceded by 68 dB pre-pulse, 5) 110 dB stimulus preceded by 82 dB pre-pulse, and 6) 120 dB stimulus preceded by 82 dB pre-pulse. Testing consisted of eight presentations of each of the six trial types in a randomly assigned order. This resulted in a total of 48 experimental trials with inter-trial intervals varying randomly from 10-30 seconds. The entire testing period lasted approximately 27 minutes. These stimuli and pre-pulse values were chosen based on their wide use in the literature and previous research that has shown significant effects of nicotine, MK-801, and other manipulations on ASR and PPI (Acri, Grunberg, & Morse, 1991; Acri et al., 1994; Bakshi et al., 1994; Bakshi & Geyer, 1995; Faraday, 2000).

The equipment used to measure ASR was recalibrated between experiments, resulting in a different baseline platform sensitivity for each set of animals. This procedure does not affect the analysis of data within each of the three individual experiments. It does, however, limit comparisons of data

between any of the discrete experiments. It should be noted that ASR values for these experiments differ significantly, but this does not affect the interpretation of PPI. PPI is expressed as a percent reduction in ASR, not an absolute ASR value.

Drug Administration

Nicotine at one of two different doses (0.1 or 0.5 mg/kg) or physiological saline (0.9% saline) was administered to subjects via a subcutaneous injection (SC) located on the animal's dorsal side, between the shoulder blades. These dosages were chosen based on previous work that reported significant effects of nicotine on ASR and PPI using nicotine dosages in this range (Acri et al., 1994; Popke, 1996) as well as ongoing research in our laboratory (Elliott, Phillips, Faraday, & Grunberg, 2001; Elliott, Faraday, & Grunberg, 2002). These two particular dosages were selected to represent a mid-range of doses that would likely be effective based upon previous research that has shown them to significantly affect various behavioral measures (Elliott et al., 2001; Elliott et al., 2002). Nicotine solutions were prepared using nicotine dihydrochloride with physiological saline as a vehicle and were calculated and expressed in terms of the nicotine base. Nicotine solutions were pH-adjusted to match the pH of physiological saline (6.5-7.0) using a 1 molar disodium phosphate (Na_2HPO_4) solution.

Dizocilpine (MK-801) (0.15 mg/kg) or physiological saline was administered via an intra-peritoneal (IP) injection 5 minutes post-nicotine

injection. This dosage was chosen based on previously reported findings that this dosage effectively increases ASR and decreases PPI in Sprague-Dawley rats (Bakshi et al., 1994; Bakshi & Geyer, 1995; Feifel et al., 1999). The MK-801 solution was prepared using dizocilpine maleate (Sigma-Aldrich/RBI, St. Louis, MO) with physiological saline as a vehicle and was calculated and expressed in terms of the dizocilpine base.

Data Collection Procedure

Subjects were gentled for two days following arrival from the supplier to minimize stress effects during testing. They were then acclimated three times to the equipment in a procedure in which subjects were placed in the acoustic startle apparatus and exposed to noise stimuli for approximately 24 minutes. The acclimation period is intended to minimize any effect of exposure to novel stimuli on the acoustic startle response (Faraday & Grunberg, 2000). Two days after the final acclimation exposure, baseline measures of ASR and PPI were collected.

Using the baseline ASR and PPI data, as well as current body weight data, each subject was assigned to an experimental condition, either saline control or one of two dosages (0.1 or 0.5 mg/kg) of nicotine. Subjects were assigned to ensure that each experimental group had comparable initial body weight as well as comparable baseline ASR and PPI measures.

Experimental data were collected on the second day following baseline testing.   Subjects received a subcutaneous injection of either nicotine (0.1 or 0.5

mg/kg) or saline followed 5 minutes later by an intraperitoneal injection of either MK-801 (0.15 mg/kg) or saline. After a 10-minute waiting period, animals were placed in Plexiglas holders and into the individual startle chambers. Acoustic startle amplitude data were then collected.

Data Analytic Strategy

Responses for individual animals were averaged across the eight trials within each of the six trial types. The acoustic startle response (ASR) was measured directly as the magnitude of platform displacement for trials with startle stimuli but no pre-pulses. Percent pre-pulse inhibition (PPI) was determined by subtracting the amplitude of trials with startle stimuli preceded by pre-pulses from the amplitude of trials with equivalent startle stimuli, but no pre-pulses. This difference was then converted to a percentage through the following equation: [(amplitude of trial without pre-pulse) – amplitude of trial with pre-pulse) / (amplitude of trial without pre-pulse)] (Acri, 1992; Acri, 1994). These calculations were performed for each animal on every day of data collection.

Data from the acute drug administration day, both ASR in the form of startle amplitude and PPI expressed as percent pre-pulse inhibtion, were analyzed using multivariate analyses of variance (MANOVAs) (Acri, 1992; Faraday, O'Donoghue, & Grunberg, 1999). Univariate analyses of variance (ANOVAs) were performed when significant multivariate effects were found. All tests were two-tailed, with alpha ≤ 0.05.

Results

Acoustic Startle (ASR) and Pre-pulse Inhibition (PPI)

Mean acoustic startle reflex amplitudes for each group are presented in Figures 1a-1b. Figures 1c-1f display mean percent pre-pulse inhibition for all groups.

The data for the acoustic startle amplitude to both 110 dB and 120 dB violated the criteria of homogeneity of variance according to Levene's Test of Equality of Error Variances. These data were subsequently subjected to log transformation that corrected the homogeneity of variance problems. The results presented for the acoustic startle amplitude are based on the log-transformed data.

Multivariate tests revealed that there was no significant effect of nicotine on the startle amplitude or percent pre-pulse inhibition at any of the startle or pre-pulse parameters. There was a significant main effect of MK-801 on startle amplitude [$F(2,54) = 22.63$, $p<0.05$] and on percent pre-pulse inhibition [$F(4,52) = 10.10$, $p<0.05$]. Subsequent ANOVAs were performed for each trial type.

Startle Amplitude to 110 dB. Animals receiving MK-801 startled more than those that received saline, [$F(1,55) = 36.10$, $p<0.05$].

Startle Amplitude to 120 dB. Administration of MK-801 decreased the amount of PPI relative to animals treated with saline, [$F(1,55) = 41.71$, $p<0.05$].

Percent PPI to a startle of 110 dB and a pre-pulse of 68 dB. Administration of MK-801 decreased the amount of PPI relative to saline controls, [$F(1,55) = 23.95$, $p<0.05$].

Percent PPI to a startle of 120 dB and a pre-pulse of 68 dB.   Animals receiving MK-801 had less PPI than saline-treated animals, [$F_{(1,55)} = 25.75$, $p<0.05$].

Percent PPI to a startle of 110 dB and a pre-pulse of 82 dB. Administration of MK-801 decreased the amount of PPI relative to saline controls, [$F_{(1,55)} = 32.11$, $p<0.05$.

Percent PPI to a startle of 120 dB and a pre-pulse of 82 dB.   MK-801-treated animals exhibited less PPI than saline controls, [$F_{(1,55)} = 23.30$, $p<0.05$].

Discussion

Experiment 1 investigated the effects of nicotine on MK-801-induced sensorimotor gating deficits, as measured by ASR amplitude and percent PPI. More specifically, this experiment was designed to investigate the effects of nicotine on an animal model of the attentional difficulties associated with schizophrenia in humans.

The hypothesis that administration of MK-801 would increase startle amplitude was supported. Subjects receiving MK-801 exhibited significantly greater startle amplitudes to the 110 dB and 120 dB startle stimuli compared with animals not receiving MK-801. This finding is consistent with previous reports that MK-801 increases startle amplitude (Bakshi et al., 1994; Varty & Higgins, 1995; Bakshi & Geyer, 1995; Feifel et al., 1999; Varty et al., 1999).

The hypothesis that treatment with MK-801 would decrease sensorimotor gating as measured by PPI also was confirmed.   Animals receiving MK-801

displayed significantly smaller percent PPI to all startle and pre-pulse stimuli relative to those animals that were not administered MK-801. This finding also is consistent with previous work reporting decreased PPI in rats receiving MK-801 (Bakshi et al., 1994; Varty & Higgins, 1995; Bakshi & Geyer, 1995; Feifel et al., 1999; Varty et al., 1999).

The hypothesis that nicotine administration would significantly decrease startle amplitude was not confirmed in this experiment. This finding is partially inconsistent with previous research in the area. Acri et al. (1994) reported that acute administration of nicotine at doses of 0.5 mg/kg and higher significantly decreased startle amplitude relative to saline controls, but 0.1 mg/kg nicotine had no significant effect on startle.

The hypothesis that nicotine would increase percent PPI also was not confirmed. This finding is partially inconsistent with previous research. Acri et al. (1994) reported that acute administration of nicotine at low doses resulted in an increase in percent PPI relative to saline controls, but results were not significant at dosages above 0.01 mg/kg. Popke (1996) reported that acute nicotine increased PPI in rats receiving nicotine (0.5 mg/kg) and exposed to a 122 dB startle stimulus (Popke, 1996).

Finally, the hypothesis that there would be a significant Nicotine X MK-801 interaction was not confirmed. There were no significant interactions of nicotine and MK-801 for either ASR or percent PPI for any of the startle and pre-pulse parameters tested.

The failure of nicotine to alter the attentional effects of MK-801 appears to be a real phenomenon and not a result of power. Although the observed power was low, observed effect sizes for these results also were low (partial eta-squared values $\leq$ 0.065 for all variables), indicating that even with an increase in power, a significant effect of nicotine and significant nicotine x MK-801 interactions would be difficult to find.

However, it could be that the nicotine dosages used in Experiment 1 were not appropriate to alter the attentional effects of MK-801. Acri et al. (1994) used a wide spectrum of acute nicotine dosages ranging from 0.001 to 5.0 mg/kg nicotine while the present experiment used only two mid-range dosages. These dosages, 0.1 and 0.5 mg/kg nicotine, may have been too high or too low to produce the behavioral changes that were anticipated. These significant behavioral changes resulting from nicotine administration, including increased PPI, may be necessary if nicotine is to minimize or reverse the sensorimotor deficits caused by MK-801.

Experiment 2 addresses these dosage issues. The effects of both a lower, 0.01 mg/kg, and a higher, 1.0 mg/kg, dose of nicotine on ASR and PPI in MK-801-treated animals were examined in Experiment 2.

EXPERIMENT 2

<u>Overview</u>

The purpose of Experiment 2 was to examine the effects of acute nicotine on the acoustic startle response (ASR) amplitude and pre-pulse inhibition (PPI) of acoustic startle in animals treated with MK-801, an NMDA receptor antagonist in a 2 (0 or 0.15 mg/kg MK-801) x 3 (0, 0.01, or 1.0 mg/kg nicotine) factorial design. Previous studies of animals treated with MK-801 have reported increased ASR amplitude and decreased PPI, suggesting that MK-801 may interrupt attentional processes and cause deficits in sensorimotor gating (Bakshi et al., 1994; Varty & Higgins, 1995; Feifel et al., 1999; Varty et al., 1999).

Mid-level acute doses of nicotine, 0.1 and 0.5 mg/kg, did not affect MK-801- induced sensorimotor deficits in Experiment 1. Experiment 2 was designed to examine the effects of a lower and a higher dose of nicotine. Based on previous studies of the effects of acute nicotine on ASR and PPI (Acri et al., 1994; Popke, 1996), it was hypothesized that administration of 0.01 mg/kg or 1.0mg/kg acute nicotine would counter MK-801 induced deficits.

Following acclimation and baseline exposures to the acoustic startle equipment, animals were assigned to experimental conditions. On the day of testing, all animals received injections of nicotine (0, 0.01, or 1.0 mg/kg) and MK-801 (0 or 0.15 mg/kg) before being exposed to the startle and pre-pulse stimuli. Trials composed of a startle stimulus, 110 dB or 120 dB, with and without pre-pulse stimuli, 68 dB or 82 dB, were presented in a random order. ASR amplitude

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was measured using an interfaced computer while PPI was determined

mathematically using ASR data from trials with and without pre-pulse stimuli.

<u>Hypotheses</u>

Hypothesis 1: MK-801 administration would significantly increase acoustic startle amplitude.

Rationale: Several studies have demonstrated a significant increase in the acoustic startle reflex of rats following administration of MK-801 in the dose range of 0.1 – 0.3 mg/kg (Bakshi et al., 1994; Varty & Higgins, 1995; Feifel et al., 1999; Varty et al., 1999). In addition, this dose of MK-801 was effective in significantly increasing startle amplitude in Experiment 1 of this series.

Hypothesis 2: MK-801 administration would significantly decrease percent pre-pulse inhibition (PPI).

Rationale: A significant decrease in PPI has been found following administration of MK-801 in the dose range of 0.1 – 0.3 mg/kg in multiple studies (Bakshi et al., 1994; Varty & Higgins, 1995; Feifel et al., 1999; Varty et al., 1999). In addition, this dose of MK-801 was effective in significantly decreasing percent PPI in Experiment 1 of this series.

Hypothesis 3: Nicotine, at the 0.01 mg/kg dose, will significantly increase the acoustic startle amplitude.

Rationale: Acri et al. (1994) reported an increase in the acoustic startle reflex of animals treated with acute nicotine at a dose of 0.01 mg/kg.

Hypothesis 4: Nicotine, at the 1.0 mg/kg dose, would significantly decrease the acoustic startle amplitude.

Rationale: Acri et al. (1994) reported a decrease in the acoustic startle reflex of animals treated with acute nicotine at doses of 0.5 mg/kg and higher, to include 1.0 mg/kg.

Hypothesis 5: Nicotine, at the 0.01 mg/kg dose, would significantly increase percent PPI.

Rationale: Acri et al. (1994) and Popke (1996) both reported findings of significant increases in percent PPI in animals treated acutely with 0.01 mg/kg of nicotine.

Hypothesis 6: There would be a significant Nicotine X MK-801 interaction for percent PPI.

Rationale: Based upon previous findings that nicotine increases PPI at an acute dose of 0.01 mg/kg while MK-801 decreases it, it was hypothesized that an interaction would result. Animals receiving both the low dose of nicotine and MK-801 should have greater PPI than those receiving MK-801 alone. Nicotine was hypothesized to effectively minimize or reverse the effects of MK-801 on PPI.

Methods

Subjects

Subjects were 60 different adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA). Subject selection was performed using the same criteria as in Experiment 1. The cell size of n=10 was based upon previous research that reported significant effects of acute nicotine (Acri, 1992; Acri et al., 1994; Popke, 1996) and MK-801 (Al-Amin & Schwarkopf, 1996; Varty, Bakshi, & Geyer, 1999) on ASR and PPI. Animals were aged 50-52 days and weighed 225-250 g at the beginning of the experiment. All subjects were pair-housed in standard polypropylene rectangular cages on hardwood chip bedding (Pine-Dri) with unlimited access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained on a 12-hour reverse light/dark cycle (lights on at 1730 hours) at a temperature of 23°C and 50 percent relative humidity.

Equipment

The acoustic startle reflex (ASR) and pre-pulse inhibition (PPI) were measured using the same equipment as described previously in Experiment 1. All procedures and parameters, including startle and pre-pulse stimuli as well as trial specifications, were identical to those in Experiment 1.

Drug Administration

Nicotine at one of two different dosages (0.01 mg/kg or 1.0 mg/kg) or physiological saline (0.9% saline) was administered to subjects via a subcutaneous injection (SC) on the animal's dorsal side, between the shoulder blades. Nicotine solutions were prepared using nicotine dihydrochloride with physiological saline as a vehicle and were calculated and expressed in terms of nicotine base. Nicotine solutions were pH-adjusted to match the pH of physiological saline (6.5-7.0) using a 1M disodium phosphate (Na_2HPO_4) solution.

Dizocilpine (MK-801) (0.15 mg/kg) or physiological saline was administered via an intra-peritoneal (IP) injection 5 minutes post-nicotine injection. The MK-801 solution was prepared using dizocilpine maleate (Sigma-Aldrich/RBI, St. Louis, MO) with physiological saline as a vehicle and was calculated and expressed in terms of dizocilpine base.

Data Collection Procedure

Subjects underwent gentling, acclimation, and baseline procedures identical to those previously described in Experiment 1.

Using the baseline ASR and PPI data, as well as current body weight data, each subject was assigned to an experimental condition, either saline control or one of two dosages (0.01 or 1.0 mg/kg) of nicotine. Subjects were assigned to ensure that each experimental group had comparable initial body weight.

Experimental data were collected on the second day following baseline testing. Subjects received a subcutaneous injection of either nicotine (0.01 or 1.0 mg/kg) or saline followed 5 minutes later by an intraperitoneal (IP) injection of either MK-801 (0.15 mg/kg) or saline. After a 10-minute waiting period, animals were placed in Plexiglas holders and into the individual startle chambers. Acoustic startle amplitude data were then collected.

Data Analytic Strategy

The data analytic strategy for Experiment 2 was identical to that of Experiment 1 including the treatment of the data and calculations to transform startle amplitude to percent PPI

Data from the acute drug administration day, both ASR in the form of startle amplitude and PPI expressed as percent pre-pulse inhibtion, were analyzed using multivariate analyses of variance (MANOVAs) (Acri, 1992; Faraday, O'Donoghue, & Grunberg, 1999). Univariate analyses of variance (ANOVAs) were performed when significant multivariate effects were found. All tests were two-tailed, with alpha $\leq$ 0.05, as in Experiment 1.

<u>Results</u>

Acoustic Startle (ASR) and Pre-pulse Inhibition (PPI)

Mean acoustic startle reflex amplitudes for each group are presented in Figures 2a-2b. Figures 2c-2f display mean percent pre-pulse inhibition for all groups.

Multivariate analyses revealed that there was no significant effect of nicotine on the startle amplitude or percent pre-pulse inhibition at any of the startle or pre-pulse parameters. There was a significant trend towards a main effect of MK-801 on startle amplitude [$F(2,53) = 3.13$, $p=0.052$] and a significant effect of MK-801 on percent pre-pulse inhibition [$F(4,51) = 10.14$, $p<0.05$]. There was also a significant overall nicotine X MK-801 interaction [$F(8,104) = 2.360$, $p<0.05$]. Subsequent ANOVAs were performed for each trial type.

Startle Amplitude to 110 dB.   Animals receiving MK-801 startled more than those that received saline, [$F(1,53) = 6.16$, $p<0.05$].

Startle Amplitude to 120 dB.   Animals receiving MK-801 startled more than those that received saline, [$F(1,53) = 4.36$, $p<0.05$].

Percent PPI to a startle of 110 dB and a pre-pulse of 68 dB. Administration of MK-801 decreased the amount of PPI relative to saline controls, [$F(1,54) = 24.49$, $p<0.05$].

Percent PPI to a startle of 120 dB and a pre-pulse of 68 dB.   MK-801-treated animals had decreased PPI compared with saline-treated animals, [$F(1,54) = 19.56$, $p<0.05$].

Percent PPI to a startle of 110 dB and a pre-pulse of 82 dB.   Animals receiving MK-801 had decreased PPI relative to saline controls, [$F(1,54) = 17.84$, $p<0.05$].

Percent PPI to a startle of 120 dB and a pre-pulse of 82 dB. Administration of MK-801 decreased the amount of PPI compared with saline control animals, [$F(1,54) = 18.10$, $p<0.05$]. Although there was no significant

effect of nicotine on PPI, there was a significant nicotine x MK-801 interaction,

$[F(2, 54) = 4.29, p<0.05]$.   When the data were split by MK-801 status, there was

no significant effect of nicotine on saline treated animals. There was, however, a

significant effect of nicotine on animals receiving MK-801, $[F(2, 27)=4.07,$

$p<0.05]$. Post-hoc analysis of the data with Tukey's HSD revealed that animals

receiving MK-801 and 1.0 mg/kg nicotine had significantly higher percent pre-

pulse inhibition than those receiving either 0.01 mg/kg nicotine or saline. There

was no significant effect of the lower nicotine dosage.

Discussion

Experiment 2 investigated the effects of two additional dosages of nicotine

on MK-801 induced sensorimotor gating deficits, as measured by the acoustic

startle reflex amplitude (ASR) and percent pre-pulse inhibition (PPI). This

experiment was designed based upon the results of Experiment 1 to examine the

effects of a lower and higher dosage of nicotine, 0.01 and 1.0 mg/kg nicotine, on

ASR and PPI.

The hypotheses that treatment with MK-801 would increase ASR

amplitude and decrease sensorimotor gating, as measured by PPI, were

confirmed.   Subjects receiving MK-801 exhibited significantly greater startle

amplitudes as well as significantly decreased PPI to all startle and pre-pulse

stimuli relative to those animals that were not administered MK-801. This finding

is consistent with previous findings that MK-801 increases startle amplitude

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(Bakshi et al., 1994; Varty & Higgins, 1995; Bakshi & Geyer, 1995; Feifel et al., 1999; Varty et al., 1999), as well as the results of Experiment 1.

The hypotheses that nicotine administration would significantly affect startle amplitude were not confirmed in this experiment. This finding was inconsistent with the hypothesis that the high dose of nicotine would *decrease* startle amplitude. Previous research has reported that Sprague-Dawley rats startle significantly *less* than saline controls when receiving nicotine acutely at the 1.0 mg/kg dose (Acri et al., 1994)

In addition, the hypothesis that nicotine would increase percent PPI was not confirmed. This finding is also inconsistent with previous research. Acri et al. (1994) and Popke (1996) reported that acute administration of nicotine at low doses, including 0.01 mg/kg, resulted in an increase in percent PPI relative to saline controls.

Finally, the hypothesis that there would be a significant Nicotine X MK-801 interaction was partially confirmed. There were no significant interactions for the ASR data. There was, however, a significant interaction of nicotine and MK-801 on PPI to a startle stimulus of 120 dB and a pre-pulse of 82 dB. Upon splitting the data by MK-801 status, there was a significant effect of nicotine on animals treated with MK-801, but not saline-treated animals. Animals that received MK-801 and 1.0 mg/kg nicotine had greater PPI than those receiving only MK-801, suggesting that nicotine may have succeeded in attenuating some of the sensorimotor gating deficits produced by MK-801. It should be noted however,

that this significant interaction occurred inconsistently and only for a single set of the ASR and PPI variable.

The failure of nicotine to alter the attentional effects of MK-801 on ASR and PPI appear to be real phenomenon and not a result of insufficient power in Experiment 2. Although the observed power was low, the observed effect sizes for these results also were low (partial eta-squared values $\leq$ 0.053 for all insignificant variables), indicating that even with an increase in power, a significant effect of nicotine and significant nicotine x MK-801 interactions would be difficult to find in these variables.

However, it could be that the means of nicotine administration may not have been the most effective method for this study. While the acute administration of nicotine by means of daily injections is a commonly-used model for nicotine use and smoking, the chronic administration of nicotine, typically by means of a surgically-implanted osmotic mini-pump, is also often used. Unlike acute injections that provide a single bolus of drug, the mini-pumps supply a consistent infusion of nicotine over a period of several weeks. Although there is a limited amount of research on the effects of acute nicotine on ASR and PPI, the significant effects of chronically administered nicotine on these variables have been better documented in rats of different sex, age, and strain (Acri et al., 1994; Acri, Brown, Saah, & Grunberg, 1995; Faraday et al., 1999a). This chronic paradigm may be more effective in producing behavioral effects on ASR and PPI than the acute administration used in the previous two experiments. It is an

alternative model to test the effects of nicotine on sensorimotor gating deficits induced by treatment with MK-801.

The third study in this experimental series addresses these administration paradigm issues. Experiment 3 assessed the effect of chronic nicotine administration, 6 mg/kg/day, on ASR/PPI in MK-801 treated animals.

EXPERIMENT 3

<u>Overview</u>

The purpose of Experiment 3 was to examine the effects of chronic nicotine administration on the acoustic startle response (ASR) amplitude and pre-pulse inhibition (PPI) of acoustic startle in animals treated with MK-801, an NMDA receptor antagonist in a 2 (0 or 0.15 mg/kg MK-801) x 2 (0, or 6.0 mg/kg/day nicotine) factorial design. Previous studies of animals treated with MK-801 have reported increased ASR amplitude and decreased PPI, suggesting that MK-801 may interrupt attentional processes and cause deficits in sensorimotor gating (Bakshi et al., 1994; Varty & Higgins, 1995; Feifel et al., 1999; Varty et al., 1999).

The first two experiments of this series found little effect of acute nicotine administration on MK-801 induced sensorimotor deficits. Experiment 3 was designed to examine the effect of chronic nicotine administration on these deficits. Based on previous studies of the effects of chronic nicotine administration on ASR and PPI (Acri, Grunberg, & Morse, 1991; Acri et al., 1995), it was hypothesized that chronic treatment with 6 mg/kg/day of nicotine would counter these MK-801 induced deficits.

Following acclimation and baseline exposures to the acoustic startle equipment, animals were assigned to experimental conditions and osmotic minipumps containing either saline or nicotine were surgically implanted in all animals. On testing days, post-surgery days 4, 7, and 14, all animals received

injections of MK-801 (0 or 0.15 mg/kg) before being exposed to the startle and pre-pulse stimuli. Trials composed of a startle stimulus, 110 dB or 120 dB, with and without pre-pulse stimuli, 68 dB or 82 dB, were presented in a random order. ASR amplitude was measured using an interfaced computer while PPI was determined mathematically using ASR data from trials with and without pre-pulse stimuli.

Hypotheses

Hypothesis 1: MK-801 administration would significantly increase acoustic startle amplitude.

Rationale: Several studies have reported a significant increase in the acoustic startle reflex of rats following administration of MK-801 in the dose range of 0.1 – 0.3 mg/kg (Bakshi et al., 1994; Varty & Higgins, 1995; Feifel et al., 1999; Varty et al., 1999). In addition, this dosage of MK-801 significantly increased startle amplitude in Experiments 1 and 2 of this series.

Hypothesis 2: MK-801 adminstration would significantly decrease percent pre-pulse inhibition (PPI).

Rationale: A significant decrease in PPI has been reported following administration of MK-801 in the dose range of 0.1 – 0.3 mg/kg in multiple studies (Bakshi et al., 1994; Varty & Higgins, 1995; Feifel et al., 1999; Varty et al., 1999). In addition, this dosage of MK-801 significantly decreased percent PPI in Experiments 1 and 2 of this series.

Hypothesis 3: Chronically administered nicotine would significantly increase the acoustic startle amplitude.

Rationale: Acri et al. (1991; 1995) and Faraday et al. (1999a) reported an increase in the acoustic startle reflex of animals receiving chronic nicotine treatment.

Hypothesis 4: Nicotine would significantly increase percent PPI.

Rationale: Acri et al. (1995) and Faraday et al. (1999a) reported findings of significant increases in percent PPI in animals treated with chronic nicotine.

Hypothesis 5: There would be a significant Nicotine X MK-801 interaction for percent PPI.

Rationale: Based upon previous findings that chronic nicotine increases PPI while MK-801 decreases it, it was hypothesized that an interaction would result with animals receiving both nicotine and MK-801 demonstrating greater PPI than those receiving MK-801 alone. Nicotine was hypothesized to effectively minimize or reverse the effects of MK-801 on PPI.

Methods

Subjects

Subjects were 59 adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), the same animals used in Experiment 2. There

were 10 animals per cell with the exception of the saline/nicotine group which had 9 animals. This cell size was based upon previous research that reported significant effects of chronic nicotine (Acri et al., 1995; Faraday et al., 1999a) and MK-801 (Al-Amin & Schwarkopf, 1996; Varty, Bakshi, & Geyer, 1999) on ASR and PPI. One subject was dropped from the experiment following a difficult recovery from minipump implantation surgery. Animals were allowed a one-week washout period between experiments. Animals were aged 58-60 days and weighed 300-350 g at the beginning of the experiment. All subjects were pair-housed in standard polypropylene rectangular cages on hardwood chip bedding (Pine-Dri) with unlimited access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained on a 12-hour reverse light/dark cycle (lights on at 1730 hours) at a temperature of 23°C and 50 percent relative humidity.

Equipment

The acoustic startle reflex (ASR) and pre-pulse inhibition (PPI) were measured using the same equipment as described previously in Experiment 1. All procedures and parameters, including startle and pre-pulse stimuli as well as trial specifications, were identical to those in Experiments 1 and 2.

Drug Administration and Surgical Procedure

Nicotine (6 mg/kg/day at a rate of 0.48 µl/hour) or physiological saline was administered via a model 2002 Alzet osmotic minipump (Alza Corporation, Palo

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Alto, CA). Dosages of nicotine in this range of 6 mg/kg/day have been shown in

previous studies to produce reliable behavioral effects (Grunberg, Bowen, &

Morse, 1984; Winders & Grunberg, 1990; Faraday, Scheufele, Rahman, &

Grunberg, 1999). Blood nicotine levels in rats implanted with 6.0 mg/kg/day-

delivering minipumps more closely resemble those of human smokers than do

blood nicotine levels in animals implanted with minimpumps delivering higher

dosages of nicotine (Benowitz, 1988; Winders, Grunberg, Benowitz, & Alvarez,

1998).

Dizocilpine (MK-801) (0.15 mg/kg) or physiological saline was

administered via an intra-peritoneal (IP) injection. The MK-801 solution was

prepared using dizocilpine maleate (Sigma-Aldrich/RBI, St. Louis, MO) with

physiological saline as a vehicle and was calculated and expressed in terms of

the dizocilpine base.

Animals underwent a one-week washout period following their use in

Experiment 2 prior to mini-pump implantation.   MK-801 has an elimination half-

life in rats of approximately 87 ± 8 minutes (Schwartz & Wasterlain, 1991) in rats.

Nicotine has an estimated plasma half-life in rats ranging from 0.3 hours (Sastry,

Chance, Singh, Horn, & Janson, 1995) to 1.1 hours (Kyerematen, Taylor,

deBethizy, & Vesell, 1988). Nicotine's primary metabolites, including cotinine,

cotinine-N-oxide, and allohydroxydemethylcotinine, may have half-lives of up to

24 hours (Kyerematen et al., 1988; Sastry et al., 1995).   Based upon these data,

a one-week washout period allowed for the sufficient elimination of previously

administered MK-801 and nicotine as well as their metabolites.

Surgical Procedure: Minipumps were surgically implanted in animals anesthetized using methoxyflurane (Metofane™) in a vented hood. Rats were placed individually in a covered bell jar with anesthetic-soaked gauze pads and removed from the jar when a tail pinch produced no reflex movement. A 3 x 5 cm area between the shoulder blades of each animal was shaved and cleaned with betadine. A 1-2 cm transverse incision was made within the shaved region using a pair of blunt nosed surgical scissors. Blunt dissection and spreading of the subcutaneous tissue with the scissor tips created a pocket in the skin into which the drug- or saline-filled minipump was inserted. Minipumps were positioned with the flow modulator facing the animal's head and neck region. The surgical incision was closed using two to three 9 mm surgical stainless steel wound clips with a total surgical procedure and anesthesia time of approximately 3 minutes.

Data Collection Procedure

The animals used in Experiment 3 had undergone gentling, acclimation, and baseline measures during Experiment 2. Subjects were assigned to new groups to insure that the animals in each new experimental group were approximately equal in terms of drug history from the previous experiment. A post-hoc analysis of the ASR and PPI data collected for these animals using drug history as an independent variable revealed no significant effect for drug history.

Nicotine or saline-filled minipumps were implanted in all animals on Day 1 of the drug administration phase following the one-week washout period. ASR

and PPI testing then occurred on Days 4, 7, and 14. On these days, subjects received an IP injection of MK-801 or saline. A ten-minute waiting period was then observed before the subjects were loaded into the Plexiglas holders and into the individual startle chambers. ASR and PPI data were collected.

Data Analytic Strategy

Responses for individual animals were averaged across the eight trials within each of the six trial types.   The acoustic startle response (ASR) was measured directly as the magnitude of platform displacement for trials with startle stimuli but no pre-pulses. Percent pre-pulse inhibition (PPI) was determined by subtracting the amplitude of trials with startle stimuli preceded by pre-pulses from the amplitude of trials with equivalent startle stimuli, but no pre-pulses. This difference was then converted to a percentage through the following equation: [(amplitude of trial without pre-pulse) – amplitude of trial with pre-pulse) / (amplitude of trial without pre-pulse)] (Acri, 1992; Acri, 1994). These calculations were performed for each animal for every day of data collection.

To ensure reliable drug delivery, body weight data from Days 4, 7, and 14 of Experiment 2 were analyzed by a repeated-measures analysis of covariance (ANCOVA) with baseline body weight as a covariate. Repeated measures ANOVAs were performed on all data to determine the effects of time. Data from the drug administration days (Day 4, 7 and 14) of Experiment 2, both ASR in the form of startle amplitude and PPI expressed as percent pre-pulse inhibition, were

analyzed using multivariate analyses of variance (MANOVAs) (Acri, 1992; Faraday et al., 1999a). All tests were two-tailed, with alpha $\leq$ 0.05.

<u>Results</u>

Body weight

Body weight data for Experiment 3 are presented in Figure 3s. There was a main effect of nicotine on body weight [$F_{(1,54)}$ = 12.33, $p < 0.05$]. Subjects receiving chronic nicotine administration, regardless of MK-801 status, weighed less than did their saline-treated counterparts. In addition, there was a Time x Nicotine interaction, such that the body weights of animals receiving chronic nicotine increased more slowly over time than did the saline controls [$F_{(1,54)}$ = 5.26, $p < 0.05$].

Previous research has indicated that animals receiving chronic nicotine have lower overall body weights than saline-treated animals and tend to gain weight more slowly than saline controls (Grunberg et al., 1984; Grunberg, Winders, & Popp, 1987; Winders & Grunberg, 1990). The findings of decreased body weights and slowed growth in animals receiving nicotine in this study validates the nicotine pump delivery.

Acoustic Startle (ASR) and Pre-pulse Inhibition

Mean acoustic startle reflex amplitudes for each group for each testing day are presented in Figures 3a-3f. Figures 3g-3r display mean percent pre-pulse inhibition for all groups for each testing day.

A repeated-measures ANCOVA revealed that there was a significant effect of time on the startle amplitude to 110 dB, $F(2,112) = 24.11$, $p<0.05$, as well as startle amplitude to 120 dB, $F(2,112) = 24.77$, $p<0.05$. There were no significant effects of time on any of the measures of pre-pulse inhibition. As a result, data for the two startle stimuli alone were analyzed for each of the three days of testing while the pre-pulse inhibition data were collapsed over time for purposes of data analysis.

There were no significant effects of nicotine on acoustic startle amplitude at either 110 or 120 dB. There was a significant main effect for MK-801 on startle amplitude on Day 4 [$F(2,55) = 8.41$, $p<0.05$], Day 7 [$F(2,55) = 5.56$, $p<0.05$], and Day 10 [$F(2,55) = 5.81$, $p<0.05$]. There also was a significant main effect of MK-801 on percent pre-pulse inhibition [$F(4,53) = 7.36$, $p>0.05$]. There was a trend towards a significant main effect of nicotine on percent pre-pulse inhibition [$F(4,53) = 2.32$, $p = 0.69$]. Subsequent ANOVAs were performed.

Startle Amplitude to 110 dB. Animals receiving MK-801 startled more than those that received saline on test Day 4, [$F(1,56) = 10.28$, $p<0.05$], Day 7 [$F(1,56) = 9.50$, $p<0.05$], and Day 14, [$F(1,56) = 6.23$, $p<0.05$].

Startle Amplitude to 120 dB. Animals receiving MK-801 startled more than those that received saline on test Day 4, [$F(1,56) = 17.01$, $p<0.05$], Day 7 [$F(1,56) = 8.44$, $p<0.05$], and test Day 14, [$F(1,54) = 11.64$, $p<0.05$].

Percent PPI to a startle of 110 dB and a pre-pulse of 68 dB.

Administration of MK-801 decreased the amount of PPI, [$F_{(1,56)} = 15.50$, $p<0.05$]. There was no significant effect of nicotine on pre-pulse inhibition.

Percent PPI to a startle of 120 dB and a pre-pulse of 68 dB.

Administration of MK-801 decreased the amount of PPI, [$F_{(1,56)} = 29.67$, $p<0.05$]. There was no significant effect of nicotine on pre-pulse inhibition.

Percent PPI to a startle of 110 dB and a pre-pulse of 82 dB.

Administration of MK-801 decreased the amount of PPI, [$F_{(1,55)} = 12.17$, $p<0.05$]. There was no significant effect of nicotine on pre-pulse inhibition.

Percent PPI to a startle of 120 dB and a pre-pulse of 82 dB.

Administration of MK-801 decreased the amount of PPI, [$F_{(1,55)} = 22.82$, $p<0.05$].

Discussion

Experiment 3 investigated the effects of 6 mg/kg/day of nicotine on MK-801 induced sensorimotor gating deficits, as measured by the acoustic startle reflex amplitude (ASR) and percent pre-pulse inhibition (PPI). This experiment was designed based upon the results of Experiments 1 and 2 to examine the effects of a chronic nicotine administration paradigm on ASR and PPI in animals treated with MK-801.

The hypotheses that treatment with MK-801 would increase ASR amplitude and decrease sensorimotor gating, as measured by PPI, were again confirmed. Subjects receiving MK-801 exhibited significantly greater startle

amplitudes as well as significantly decreased PPI to all startle and pre-pulse stimuli relative to those animals receiving saline. This finding is consistent with previous findings that MK-801 increases startle amplitude (Bakshi et al., 1994; Varty & Higgins, 1995; Bakshi & Geyer, 1995; Feifel et al., 1999; Varty et al., 1999), as well as the results of Experiments 1 and 2.

The hypothesis that nicotine administration would significantly increase startle amplitude was not confirmed in this experiment. Animals receiving chronic nicotine were not significantly different in terms of startle amplitude from those receiving saline for either the 110dB or 120 dB startle stimulus. This finding was not consistent with previous reports of a significant increase in startle amplitude in male Sprague-Dawley rats receiving chronic nicotine (Acri et al., 1994; Faraday et al., 1999a).

The hypothesis that chronic nicotine would significantly increase percent PPI was also not confirmed. Although there was a trend toward a significant main effect of nicotine, there were no significant effects of nicotine on PPI for any stimuli. This finding also is inconsistent with previous findings that chronic nicotine increases PPI (Faraday et al., 1999a) to similar stimuli.

The hypothesis that there would be a significant Nicotine X MK-801 interaction was not confirmed. There were no significant interactions between the two drugs for any of the stimuli or pre-pulse combinations.

Although this study utilized a chronic paradigm of nicotine administration, it was somewhat limited in the dose used. While behavioral effects have been reported using the 6 mg/kg/day dosage, more consistent findings occur with a

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higher dosage, usually 12 mg/kg/day. The 6 mg/kg/day dosage was chosen for

this experiment as the lowest chronic dosage shown to have significant effects

on measured behaviors and the dose that most produced blood nicotine levels

that more closely resemble those of human smokers. It may have been more

effective behaviorally though to use the higher 12 mg/kg/day dose to insure

consistent and significant effects.

GENERAL DISCUSSION

Schizophrenics smoke at a much higher prevalence rate than the general population, prompting speculation that this behavior may be some form of self-medication. It has been suggested that nicotine, the chief psychoactive ingredient in tobacco smoke, may attenuate certain symptoms of the disorder. Specifically, nicotine may be medicating the attentional deficits and sensorimotor gating disruptions associated with the disorder. The three experiments in this series examined the effects of nicotine on the sensorimotor gating deficits in the MK-801 animal model of schizophrenia. These experiments successfully replicated the attentional deficits produced by MK-801 administration as indexed through the acoustic startle reflex (ASR) and pre-pulse inhibition (PPI). Contrary to the hypothesized effect of nicotine, the results indicate that nicotine, at the various dosages and via the various routes administered, does not have consistent effects on the sensorimotor gating deficits produced by MK-801. Overall, this potential mechanism for nicotine's medicating properties in a model of schizophrenia was not supported.

Experiment 1 examined the effects of two acute dosages of nicotine, 0.1 mg/kg and 0.5 mg/kg, on the attentional deficits in ASR and PPI produced by MK-801 administration. Significant increases in ASR and decreases in PPI were observed in rats treated with MK-801. Nicotine had no effect on these measures and did not attenuate the disruptions caused by MK-801.

Experiment 2 examined the effects of two additional acute dosages of nicotine, 0.01 mg/kg and 1.0 mg/kg, on the attentional deficits in ASR and PPI

caused by MK-801. Significant increases in ASR and decreases in PPI were observed in rats treated with MK-801. Nicotine had no significant effects on ASR or PPI. There was a significant nicotine X MK-801 interaction for PPI to a startle stimulus of 120 dB and a pre-pulse of 82 dB, such that animals that received nicotine and MK-801 displayed greater inhibition than those that received only MK-801. However, this single nicotine X MK-801 interaction indicates that these effects of nicotine were inconsistent.

Experiment 3 examined the effects of a chronic model of nicotine administration, 6 mg/kg/day administered via a surgically implanted osmotic minipump, on the attentional deficits in ASR and PPI produced by MK-801 administration. A significant increase in ASR and decrease in PPI were once again observed in rats treated with MK-801. Nicotine had no effects on ASR or PPI. Nicotine was unable to attenuate the effects of MK-801 on ASR and PPI.

The repeated finding that MK-801 increased ASR and decreased PPI is consistent with previous research (Bakshi et al., 1994; Varty & Higgins, 1995; Feifel et al., 1999; Varty et al., 1999). The findings that nicotine administration did not produce reliable, significant effects on ASR amplitude and percent PPI was not consistent with previous research using similar dosages and routes of administration (Acri et al., 1994, 1995; Popke, 1996; Faraday et al., 1999a).

It is unlikely that study design contributed to the inability to detect nicotine effects and interactions. The acute nicotine dosages used in these experiments were consistent with those used in previous studies that found significant effects for nicotine on ASR and PPI (Acri et al., 1994; Popke, 1996). The most common

chronic nicotine dosage used in previous studies was 12 mg/kg/day (Acri et al.,

1991,1995; Faraday et al., 1999a), double the 6 mg/kg/day dosage used in this

study. The 6 mg/kg/day dosage was chosen for this experiment as the lowest

chronic dosage shown to have significant effects on measured behaviors

(Grunberg, Bowen, & Morse, 1984; Winders & Grunberg, 1990; Faraday,

Scheufele, Rahman, & Grunberg, 1999) and the dose that produces blood

nicotine levels that more closely resemble those of human smokers (Benowitz,

1988; Winders, Grunberg, Benowitz, & Alvarez, 1998). Although the 6 mg/kg/day

dosage of nicotine has previously been reported to significantly increase ASR

measures (Acri et al., 1991), the 12 mg/kg/day doseage may have been more

effective behaviorally.

The single nicotine X MK-801 interaction for the attentional measures of

ASR and PPI indicates that nicotine, in general, does not consistently attenuate

the attentional disruptions caused by MK-801 administration.

It is worth noting that the nicotine used in these experiments effectively

decreased body weight and weight gain in Experiment 3, findings consistent with

previous research. These data suggest that the inconsistent effects of nicotine

on ASR and PPI were not likely due to bad or ineffective drug.

There are some limitations to these experiments that should be

recognized when drawing conclusions. These studies relied upon a single

dependent variable, ASR and PPI, which is generally characterized as a reflexive

measure of attention. It is possible that a more complex index of attention, or a

variety of attentional measures, would be a better means to examine the effects of nicotine on these deficits.

Schizophrenics may be using cigarette smoking as a form of self-medication, but relying on a substance or substances other than nicotine to alleviate symptoms.   In addition, the use of a single dependent variable, attention, limits the generalizability of these findings in reference to the self-medication hypothesis. Nicotine, or another substance in tobacco smoke, may be affecting attention but doing so indirectly, through a mediator such as affect or anxiety, variables not measured in this study.   It is also possible that schizophrenics are not smoking to alleviate attentional problems, but to directly medicate other symptoms of schizophrenia such as affect dysregulation, thought disorders, *etc.*

In addition, schizophrenics may not be smoking as a means of self-medication. There are many potential alternative reasons why smoking occurs at such a high rate in this population. Schizophrenics, and other thought-disordered individuals, may not understand or care about the negative health consequences of smoking that tend to deter others from engaging in the behavior.   They may be smoking for purely hedonistic and pleasure-seeking reasons, inclinations that a non-disordered population is better able to control.

Finally, the MK-801 model may not be the most suitable model for studying this phenomenon. There are several other animal models of schizophrenia, both pharmacologically- and environmentally-induced, that produce similar attentional deficits (Geyer, Krebs-Thomson, Braff, & Swerdlow,

2001; Weiss & Feldon). It is possible that one of these models is more appropriate for examining the effects of nicotine on the attentional deficits characteristic of schizophrenia.

Future research in this area could proceed in several directions given the findings of the present studies. Nicotine is arguably the most important component of tobacco smoke and has varied effects on multiple behavioral indices.   It would be beneficial to further pursue the self-medication hypothesis regarding nicotine, but to examine its effects on various other symptoms of schizophrenia. Animal models, such as the learned helplessness and chronic mild stress paradigms, may mimic the affect dysregulation in schizophrenia. Similar animal models also may be used to mimic the catatonic behavior often seen in schizophrenia. Studies of nicotine administration in these models would provide insight into the potential use of nicotine to self-medicate for problems other than the attentional deficits associated with the disorder.

Many of the symptoms of schizophrenia are difficult or impossible to model in animals. For example, it is not feasible to develop and validate an animal model of delusions or hallucinations. Studies examining the effects of cigarette smoking or nicotine administration in a clinical schizophrenic population would be necessary to further examine the potential for self-medication of certain symptoms.

The high correlation between schizophrenia and tobacco use remains an important area of research regardless of the reason for the high prevalence of smoking in schizophrenics. Smoking has the same detrimental health effects in

schizophrenics as it does in the general population, making it a serious threat to their well-being. Research in this area may reveal new information about the disease (*i.e.,* potential mechanisms, treatments, *etc.*) as well as smoking in general. Although these studies suggest that nicotine derived from cigarette smoking is likely not being used by schizophrenics to self-medicate attentional problems, it is important to continue to study alternative applications of the self-medication hypothesis as well as additional potential reasons for the relationship between smoking and schizophrenia.
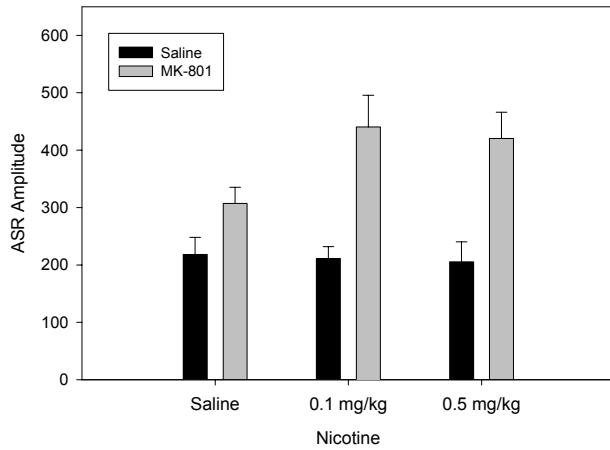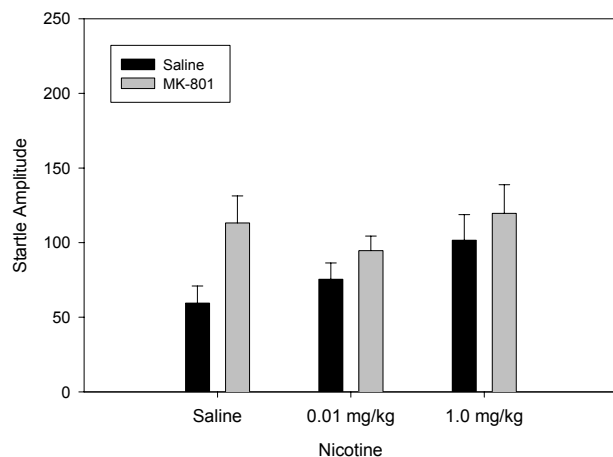
Figure 1a. Acoustic startle amplitude to a 110 dB stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0, 0.1, or 0.5 mg/kg) (Means+SEM).
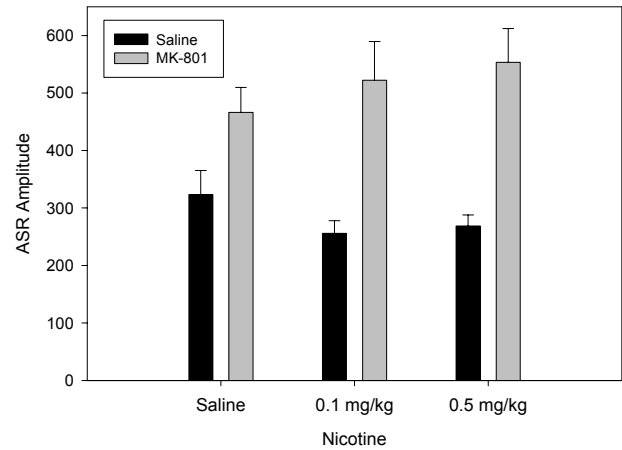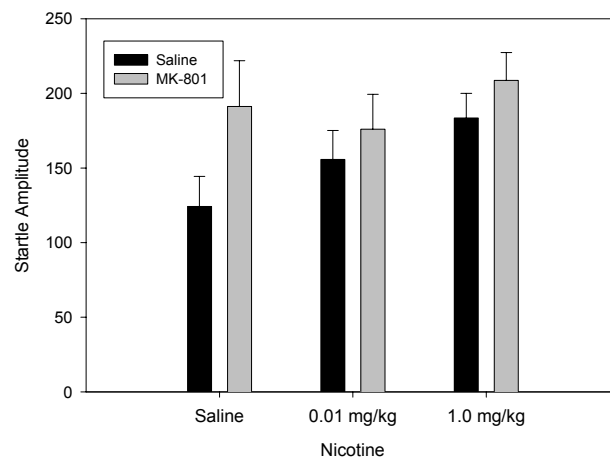

Figure 1b. Acoustic startle amplitude to a 120 dB stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0, 0.1, or 0.5 mg/kg) (Means+SEM).
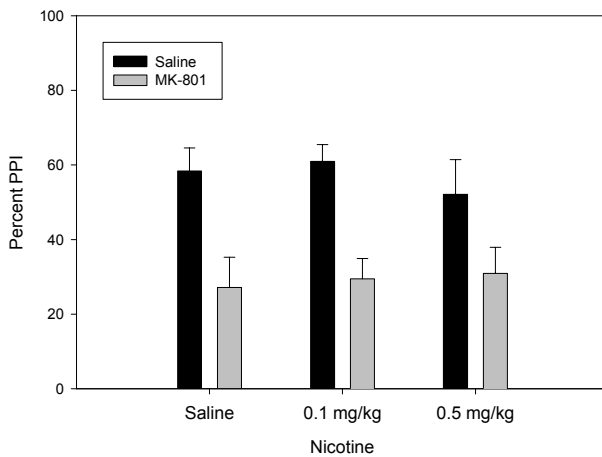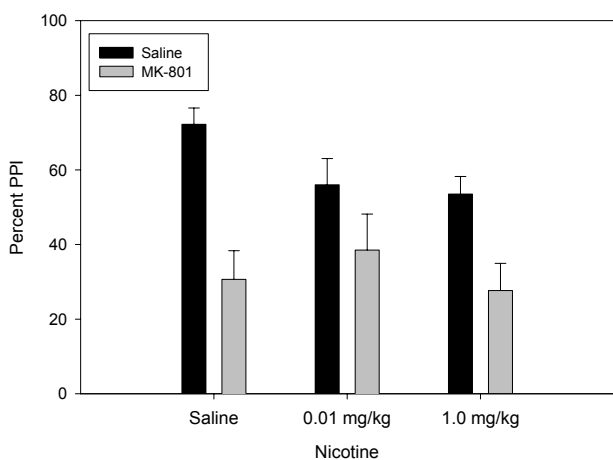

Figure 1c. Percent pre-pulse inhibition to a pre-pulse of 68 dB and a startle stimulus of 110 dB in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0, 0.1, or 0.5 mg/kg) (Means+SEM).
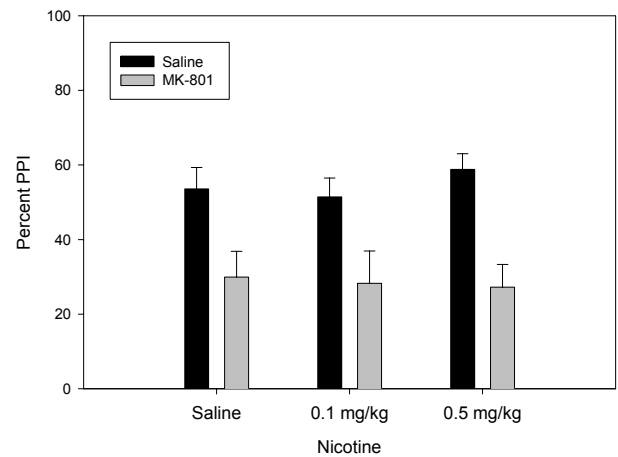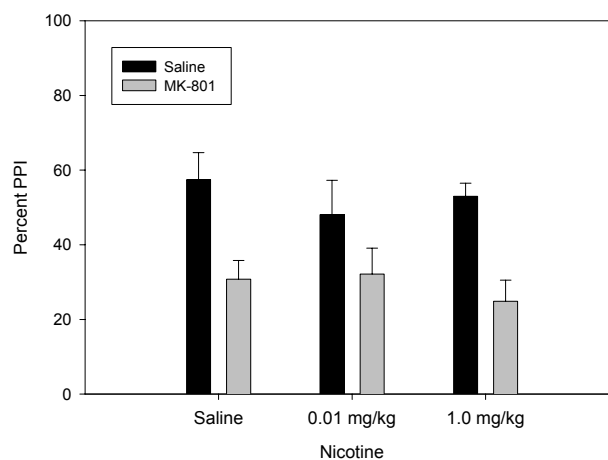

Figure 1d. Percent prepulse inhibition to a prepulse of 68 dB and a startle stimulus of 120 dB in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0, 0.1, or 0.5 mg/kg) (Means+SEM).
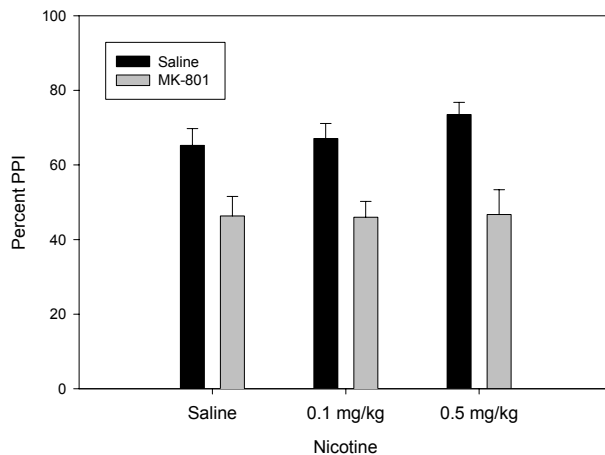
Figure 1e. Percent pre-pulse inhibition to a pre-pulse of 82 dB and a startle stimulus of 110 dB in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0, 0.1, or 0.5 mg/kg) (Means+SEM).
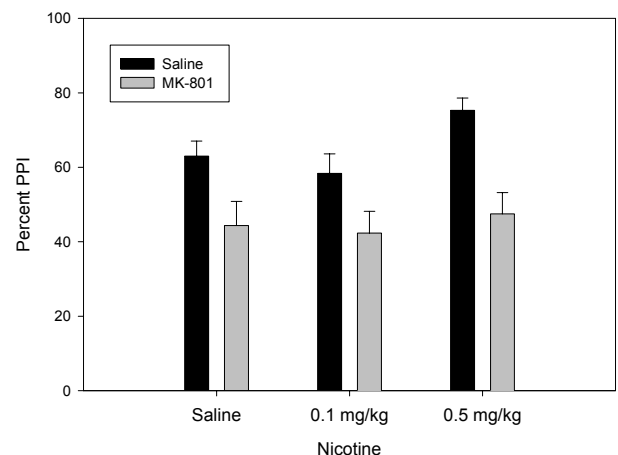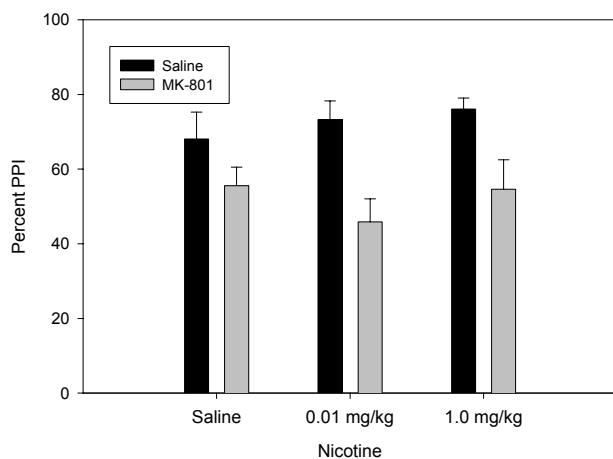

Figure 1f. Percent pre-pulse inhibition to a pre-pulse of 82 dB and a startle stimulus of 120 dB in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0, 0.1, or 0.5 mg/kg) (Means+SEM).

Figure 2a. Acoustic startle amplitude to a 110 dB stimulus in animals
receiving MK-801 (0 or 0.15 mg/kg) and nicotine
(0, 0.01, or 1.0 mg/kg) (Means +SEM).

Figure 2b. Acoustic startle amplitude to a 120 dB stimulus in animals
receiving MK-801 (0 or 0.15 mg/kg) and nicotine
(0, 0.01, or 1.0 mg/kg) (Means +SEM).

Figure 2c. Percent pre-pulse inhibition to a pre-pulse of 68 dB and a startle
stimulus of 110 dB in animals receiving MK-801 (0 or 0.15 mg/kg)
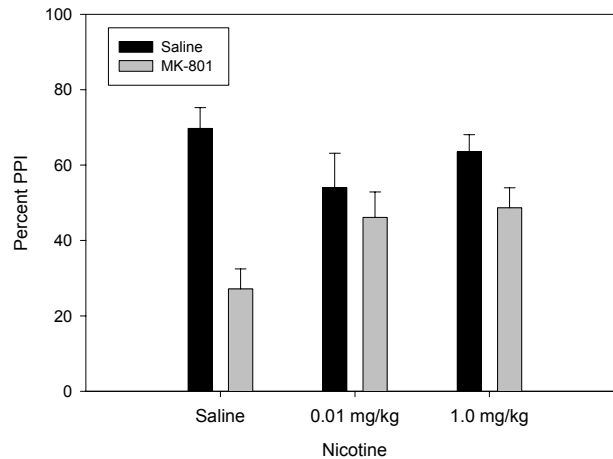and nicotine (0, 0.01, or 1.0 mg/kg) (Means +SEM).

Figure 2d. Percent pre-pulse inhibition to a pre-pulse of 68 dB and a startle
stimulus of 120 dB in animals receiving MK-801 (0 or 0.15 mg/kg)
and nicotine (0, 0.01, or 1.0 mg/kg) (Means +SEM).

Figure 2e. Percent pre-pulse inhibition to a pre-pulse of 82 dB and a startle
stimulus of 110 dB in animals receiving MK-801 (0 or 0.15 mg/kg)
and nicotine (0, 0.01, or 1.0 mg/kg) (Means +SEM).

Figure 2f. Percent pre-pulse inhibition to a pre-pulse of 82 dB and a startle
stimulus of 120 dB in animals receiving MK-801 (0 or 0.15 mg/kg)
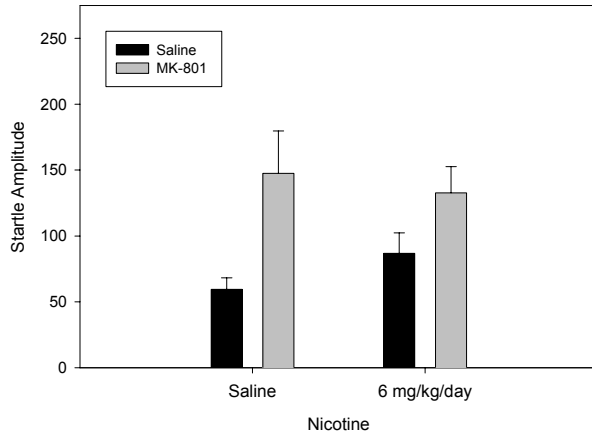and nicotine (0, 0.01, or 1.0 mg/kg) (Means +SEM).

Figure 3a. Acoustic startle amplitude on Day 1 to a 110 dB stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).
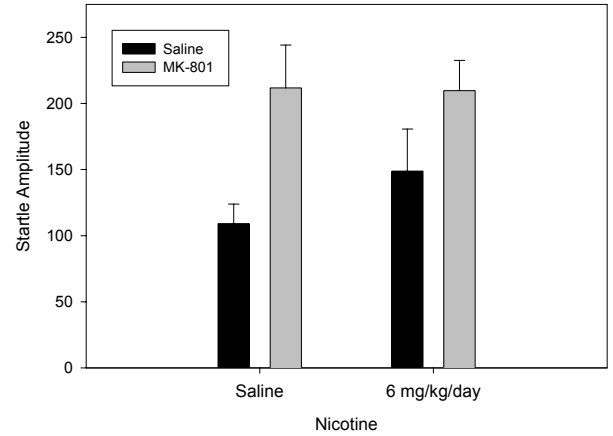

Figure 3b. Acoustic startle amplitude on Day 7 to a 110 dB stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).
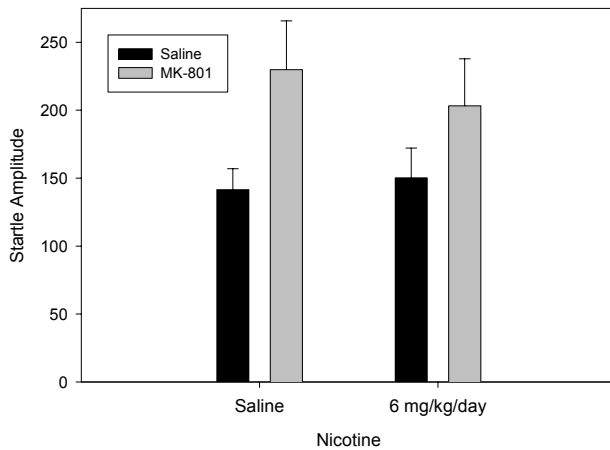

Figure 3c. Acoustic startle amplitude on Day 14 to a 110 dB stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).
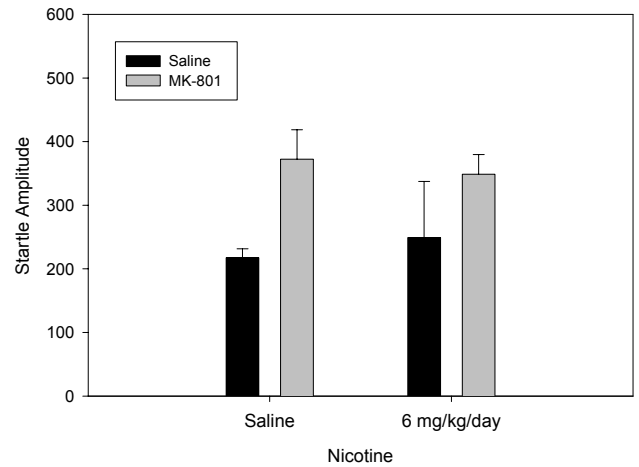

Figure 3d. Acoustic startle amplitude on Day 1 to a 120 dB stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).
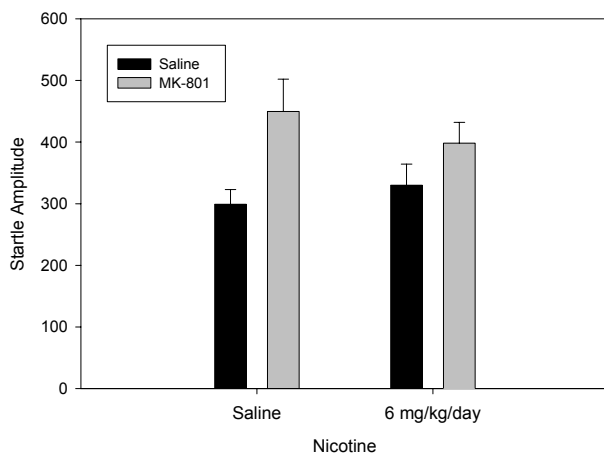

Figure 3e. Acoustic startle amplitude on Day 7 to a 120 dB stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).
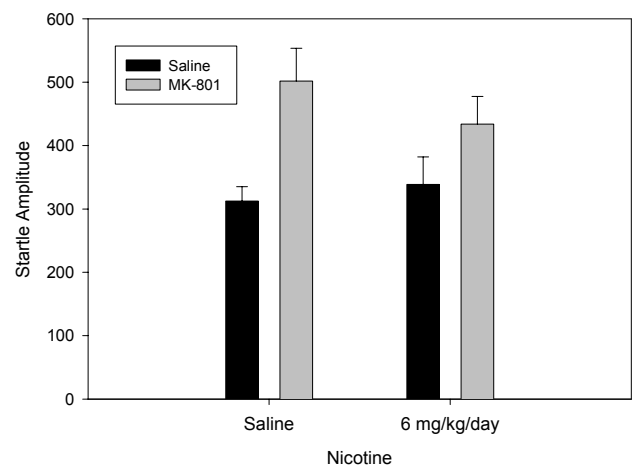

Figure 3f. Acoustic startle amplitude on Day 14 to a 120 dB stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).
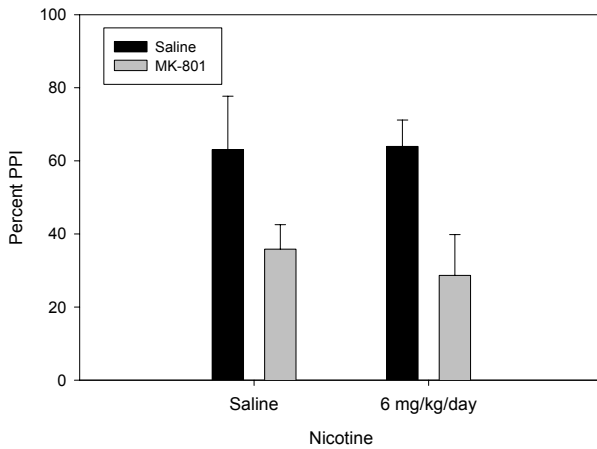
Figure 3g. Percent pre-pulse inhibition on Day 1 to a pre-pulse of 68 dB and a 110 dB startle stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).

Figure 3h. Percent per-pulse inhibition on Day 7 to a prepulse of 68 dB and a 110 dB startle stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).

Figure 3i. Percent pre-pulse inhibition on Day 14 to a pre-pulse of 68 dB and a 110 dB startle stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).

Figure 3j. Percent pre-pulse inhibition on Day 1 to a pre-pulse of 68 dB and a 120 dB startle stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).
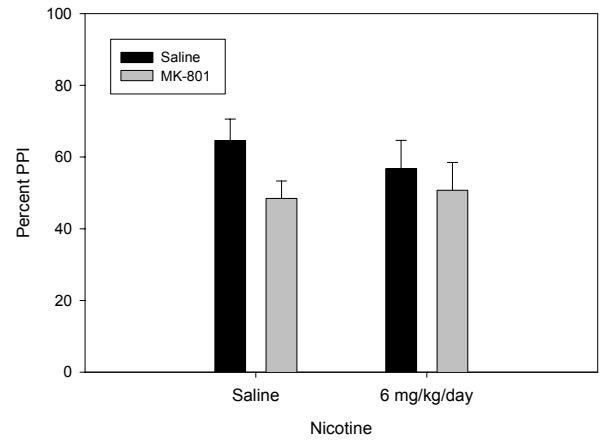

Figure 3k. Percent pre-pulse inhibition on Day 7 to a pre-pulse of 68 dB and a 120 dB startle stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).
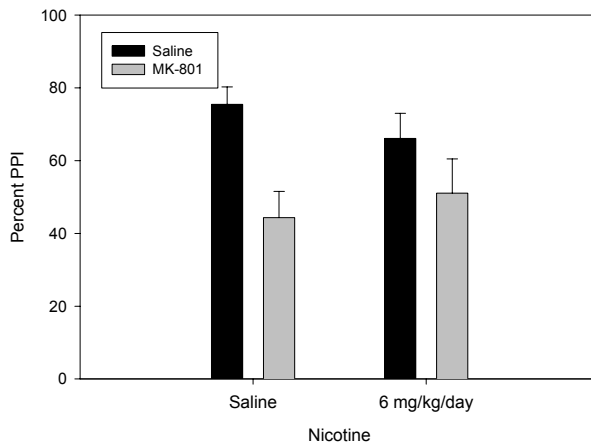

Figure 3l. Percent pre-pulse inhibition on Day 14 to a pre-pulse of 68 dB and a 120 dB startle stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).
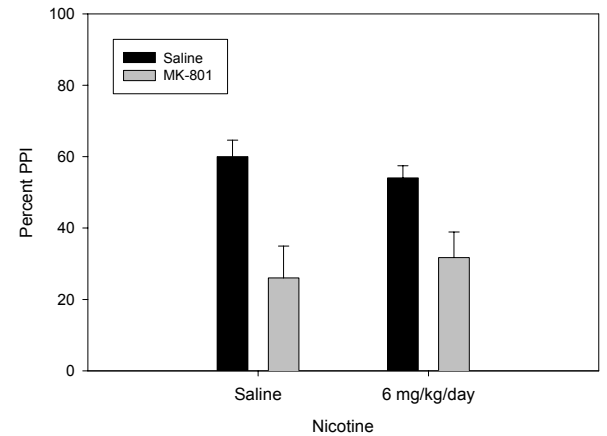
Figure 25. Percent pre-pulse inhibition on Day 1 to a pre-pulse of 82 dB and a 110 dB startle stimulus in MK-801 treated animals receiving 6.0 mg/kg/day nicotine (means and standard errors).

Figure 3n. Percent pre-pulse inhibition on Day 7 to a pre-pulse of 82 dB and a 110 dB startle stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).
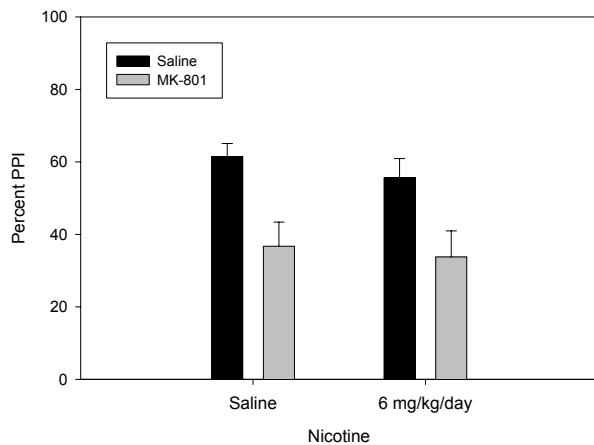

Figure 3o. Percent pre-pulse inhibition on Day 14 to a pre-pulse of 82 dB and a 110 dB startle stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).
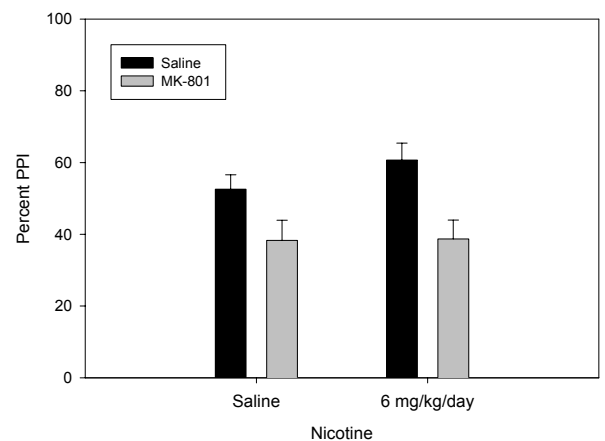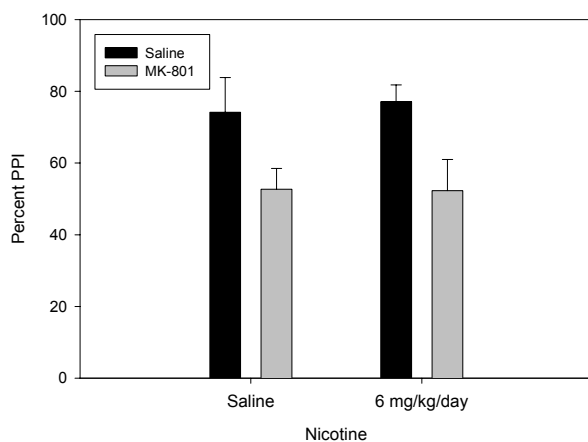

Figure 3p. Percent pre-pulse inhibition on Day 1 to a pre-pulse of 82 dB and a 120 dB startle stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).

Figure 3q. Percent pre-pulse inhibition on Day 7 to a pre-pulse of 82 dB and a 120 dB startle stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).
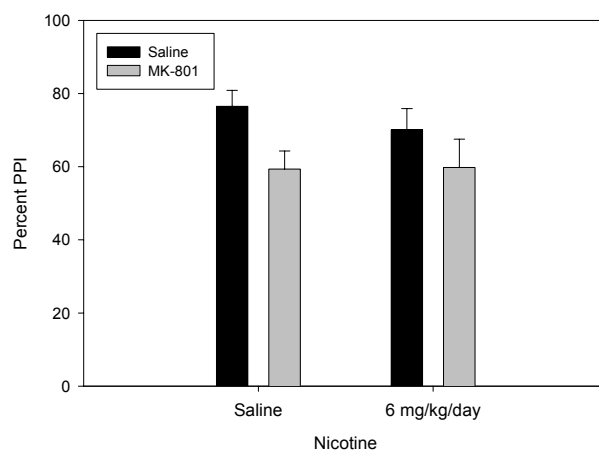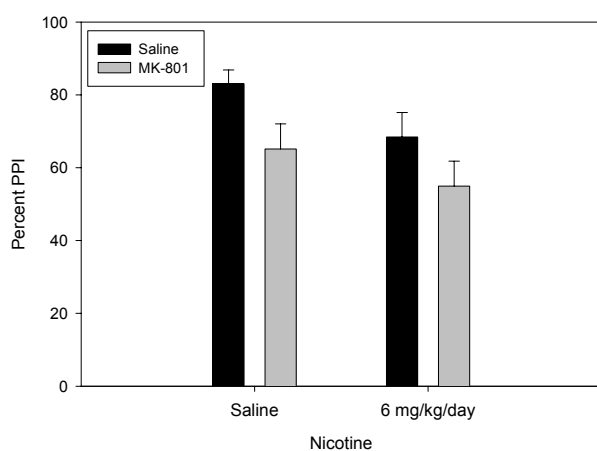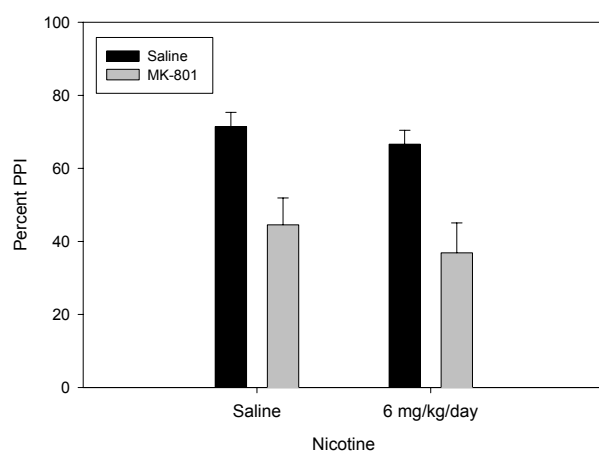

Figure 3r. Percent pre-pulse inhibition on Day 14 to a pre-pulse of 82 dB and a 120 dB startle stimulus in animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) (Means+SEM).
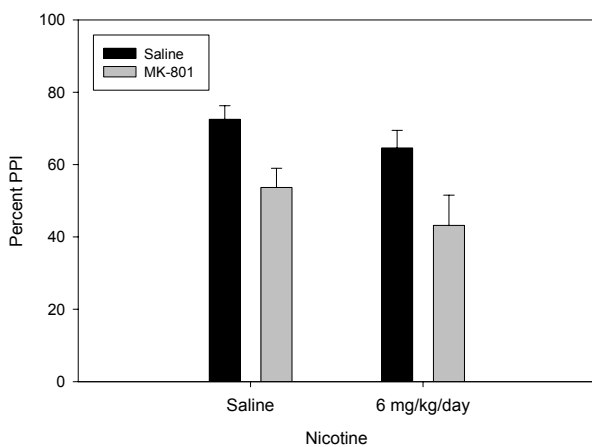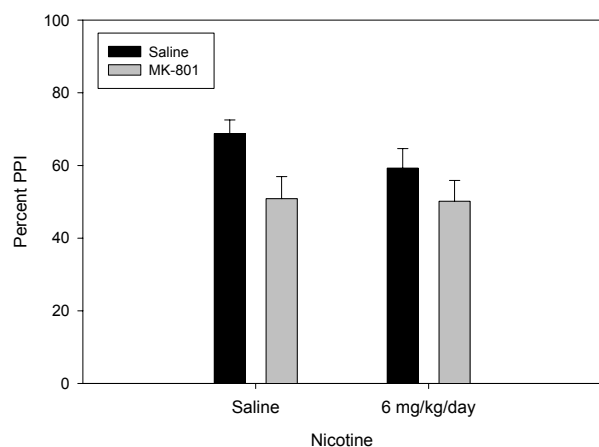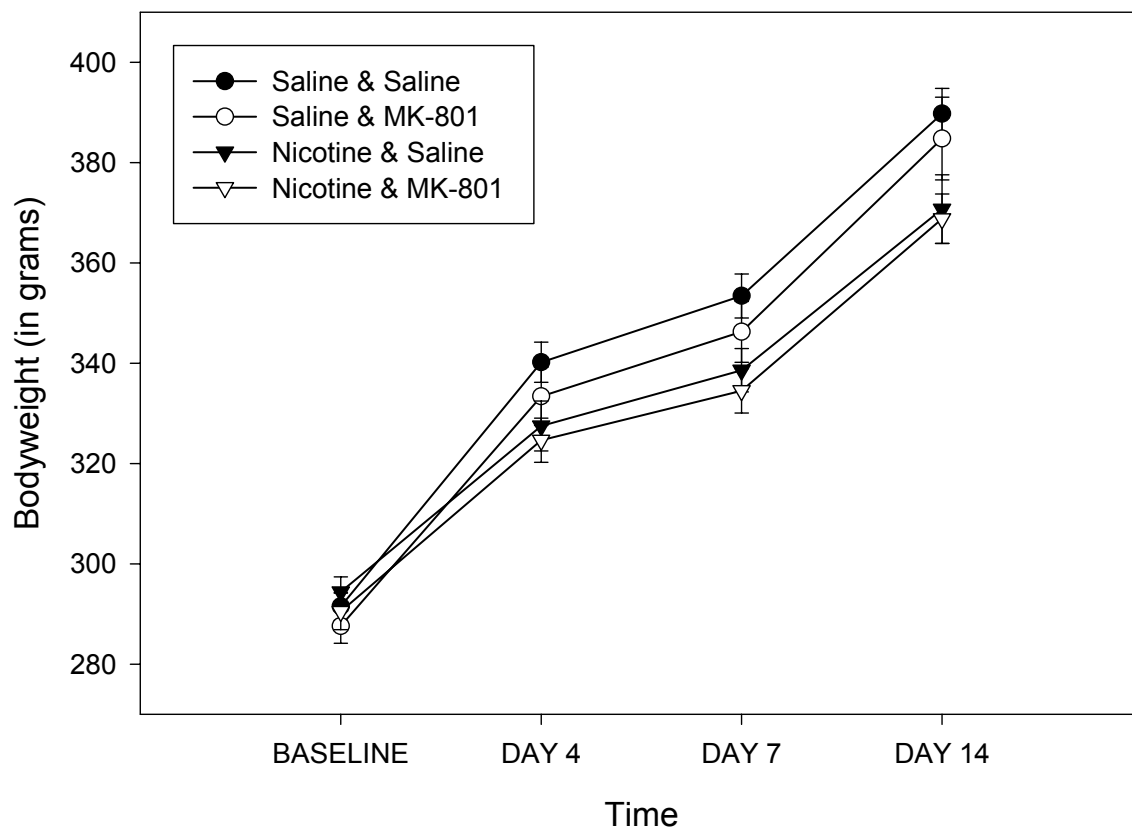
Figure 3s. Body weights for animals receiving MK-801 (0 or 0.15 mg/kg) and nicotine (0 or 6.0 mg/kg/day) at baseline (pre-treatment) and on days 4, 7, and 14 (Means$\pm$SEM).

References

Acri, J.B. (1992, unpublished doctoral dissertation). *Interactions of stress and nicotine on amplitude, pre-pulse inhibition and habituation of the acoustic startle reflex.* Bethesda, MD: Uniformed Services University of the Health Sciences.

Acri, J.B. (1994). Nicotine modulates effects of stress on acoustic startle reflexes in rats: Dependence on dose, stressor, and initial reactivity. *Psychopharmacology, 116*, 255-265.

Acri, J.B., Brown, K.J., Saah, M.I., & Grunberg, N.E. (1995). Strain and age differences in acoustic startle responses and effects of nicotine in rats. *Pharmacology, Biochemistry and Behavior, 50*, 191-198.

Acri, J.B., Grunberg, N.E., & Morse, D. E. (1991). The effects of nicotine on the acoustic startle reflex amplitude in rats. *Psychopharmacology, 104,* 244-248.

Acri, J.B., Morse, D.E., Popke, E.J., & Grunberg, N.E. (1994). Nicotine increases sensory gating measured as inhibition of the acoustic startle reflex in rats. *Psychopharmacology, 114,* 369-374.

Al-Amin, H.A., Schwarzkopf, S.B. (1996). Effects of the PCP analog dizocilpine on sensory gating: potential relevance to clinical subtypes of schizophrenia. *Biological Psychiatry, 40,* 744-755.

American Psychiatric Association. (2000). Diagnostic and statistical manual of mental disorders, fourth edition text revision (DSM-IV-TR). Washington, DC: American Psychiatric Association.

Arndt, J., & Skarsfeldt, T. (1998). Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. *Neuropsychopharmacology, 18,* 63-101.

Bakshi, V.P., & Geyer, M.A. (1995). Antagonism of phencyclidine-induced deficits in pre-pulse inhibition by the putative atypical antipsychotic olanzipine. *Psychopharmacology, 122,* 198-201.

Bakshi, V.P., Swerdlow, N.R., & Geyer, M.A. (1994). Clozapine antagonized phencyclidine-induced deficits in sensorimotor gating in the startle response. *Journal of Pharmacology and Experimental Therapeutics, 271,* 787-194.

Batel, P. (2000). Addiction and schizophrenia. *European Psychiatry, 15,* 115-122.

Benowitz, N.L. (1988). Pharmacokinectics and pharmacodynamics of nicotine. In Rand, M.J. & Thurau, K. (eds.) *The Pharmacology of Nicotine.* IRL Press Limited: Oxford, England, 3-19.

Bezchlibnyk-Butler, K.Z., & Jeffries, J.J. (2000). *Clinical handbook of psychotropic drugs.* Seattle, WA: Hogrefe & Huber Publishers.

Blanchard, J.J., Squires, D., Henry, T., Horan, W.P., Bogenschutz, M., Lauriello, J., & Bustillo, J. (1999). Examining an affect regulation model of substance abuse in schizophrenia. The role of traits and coping. *Journal of Nervous and Mental Disorders, 187,* 72-79.

Braff, D.L, & Geyer, M.A. (1990). Sensorimotor gating and schizophrenia. Human and animal model studies. *Archives of General Psychiatry, 47,* 181-188.

Braff, D.L., Grillon, C., & Geyer, M.A. (1992). Gating and habituation of the startle reflex in schizophrenic patients. *Archives of General Psychiatry, 49,* 206-215.

Braff, D.L., Swerdlow, N.R., & Geyer, M.A. (1995). Gating and habituation deficits in schizophrenia disorders. *Clinical Neuroscience, 3,* 131-139.

Braff, D.L., Stone, C., Callaway, E., Geyer, M., Glick, I., & Bali, L. (1978). Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology, 15,* 339-343.

Camp, D.E., Klesges, R.C., & Relyea, D. (1993). The relationship between body weight concerns and adolescent smoking. *Health Psychology, 12,* 24-32.

Carstens, E., Andersen, K.A., Simons, C.T., Carstens, M.I., & Jinks, S.L. (2001). Analgesia induced by chronic nicotine infusion in rats: difference by gender and pain test. *Psychopharmacology, 157,* 40-45.

Centers for Disease Control (1993). Smoking attributable mortality and years of potential life lost – United States, 1990. *Morbidity and Mortality Weekly Reports, 42,* 645-649.

Centers for Disease Control (1997). Smoking attributable mortality and years of potential life lost – United States, 1984. *Morbidity and Mortality Weekly Reports, 46,* 444-451.

Centers for Disease Control (2000). Cigarette smoking among adults – United States. *Morbidity and Mortality Weekly Reports, 48,* 881-884.

Corrigall, W. (2001). Nicotine self-administration in rats and humans. In Carroll, Marilyn E. (Ed); Overmier, J. Bruce (Ed)., *Advancing human welfare through behavioral science.* Washington, DC: American Psychological Association, 127-138.

Dalack, G.W., Healy, D.J., & Meador-Woodruff, J.H. (1998). Nicotine dependence in schizophrenia: clinical phenomenon and laboratory findings. *American Journal of Psychiatry, 155,* 1490-1501.

Davis, M. (1984). The mammalian startle response. In R. Eaton (ed.) *Neural mechanisms of startle behavior.* New York: Plenum Press, pp. 287-351.

de Leon, J., Dadvand, M., Canuso, C., White, A.O., Stanilla, J.K., & Simpson, G.M. (1995). Schizophrenia and smoking: an epidemiological survey in a state hospital. *American Journal of Psychiatry, 152,* 453-455.

Della Casa, V., Hofer, I., Weiner, I., & Feldon, J. (1998). The effects of smoking on acoustic prepulse inhibition in healthy men and women. *Psychopharmacology, 137,* 362-268.

Dixon, L., Haas, G., Weiden, P.J., Sweeney, J., & Frances, A.J. (1991). Drug abuse in schizophrenic patients: clinical correlates and reasons for use. *American Journal of Psychiatry, 148,* 224-230.

Dixon, L., Lehman, A., & Levine, J. (1995). Conventional antipsychotic medications for schizophrenia. *Schizophrenia Bulletin, 21,* 567-577.

Elliott, B.M., Faraday, M.M., & Grunberg, N.E. (2002). Nicotine's anxiolytic effects differ in adolescent and adult rats. Presented at the Society for Research on Nicotine and Tobacco, Savannah, GA.

Elliot, B.M., Phillips, J.M., Faraday, M.M., & Grunberg, N.E. (2001). Effects of nicotine on motor and social behavior in adolescent and adult, male and female rats. Presented at the Society for Research on Nicotine and Tobacco, Seattle, WA.

Faraday, M.M. (2000, unpublished doctoral dissertation). *The role of sex and strain in behavioral and biologic stress response in rats.* Bethesda, MD: Uniformed Services University of the Health Sciences.

Faraday, M.M., & Grunberg, N.E. (2000). The importance of acclimation in acoustic startle amplitude and pre-pulse inhibition testing in male and female rats. *Pharmacology, Biochemistry, and Behavior, 66,* 375-381.

Faraday, M.M., O'Donoghue, V.A., & Grunberg, N.E. (1999a). Effects of nicotine and stress on startle amplitude and sensory-gating in Long-Evans rats. *Pharmacology, Biochemistry and Behavior, 61*, 281-289.

Faraday, M.M., Scheufele, P. M., Rahman, M.A., & Grunberg, N.E. (1999b). Effects of chronic nicotine administration on locomotion depend on rat sex and housing. *Nicotine and Tobacco Research, 1*, 143-151.

Feifel, D., Reza, T.L., Wustro, D.J., & Davis, M.D. (1999). Novel anti-psychotic-like effects on pre-pulse inhibition of startle produced by a neurotensin agonist. *Journal of Pharmacology and Experimental Therapeutics, 288*, 710-713.

Foulds, J., Stapleton, J., Swettenham, J., Bell, N., McSorley, K., & Russel, M.A. (1996). Cognitive performance effects of subcutaneous nicotine in smokers and never-smokers. *Psychopharmacology, 127,* 31-38.

Frith, C. (1971). Smoking behaviour and its relation to the smoker's immediate experience. *British Journal of Social and Clinical Psychology, 10,* 73-78.

Gerlach, J. (1999). The continuing problem of extrapyramidal symptoms: strategies for avoidance and effective treatment. *Journal of Clinical Psychiatry, 60, Suppl 23,* 20-24.

Geyer, M.A., & Braff, D.L. (1987). Startle habituation and sensorimotor gating in schizophrenia and related animal models. *Schizophrenia Bulletin, 13,* 643-668.

Geyer, M.A., Krebs-Thomson, K., Braff, D.L., & Swerdlow, N.R. (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology, 156,* 117-154.

Goff, D.C., Hendersen, D.C., & Amico, E. (1992). Cigarette smoking in schizophrenia: relationship to psychopathology and medication side effects. *American Journal of Psychiatry, 149,* 1189-1194.

Grunberg, N. E. (1990). The inverse relationship between tobacco use and bodyweight. In Kozlowski, Lynn T. (Ed); Annis, Helen M. (Ed); et al. (1990). *Research advances in alcohol and drug problems*, Vol. 10. New York, NY, US: Plenum Press, pp. 273-315.

Grunberg, N.E., Bowen, D.J., Maycock, V.A., & Nespor, S. (1985). The importance of sweet taste and caloric content in the effects of nicotine on specific food consumption. *Psychopharmacology, 87,* 198-203.

Grunberg, N.E., Bowen, D.J., & Morse, D.E. (1984). Effects of nicotine on bodyweight and food consumption in rats. *Psychopharmacology, 83,* 93-98.

Grunberg, N.E., Winders, S.E., & Popp, K.A. (1987). Sex differences in nicotine's effects of consummatory behavior and body weight in rats. *Psychopharmacology, 91,* 221-225.

Hafner, H., an der Heide, W., Behrens, S., Gattaz, W.F., Hambrecht, M., Loffler, W., Marurer, K., Munk-Jorgensen, P., Nowotny, B., Riecher-Rossler, A., & Stein, A. (1998). Causes and consequences of the gender difference in age at onset of schizophrenia. *Schizophrenia Bulletin, 24,* 99-113.

Helzer, J.E., & Pryzbeck, T.R. (1988). The co-occurrence of alcoholoism and other psychiatric disorders in the general population and its impact treatment. *Journal of Studies on Alcohol, 49,* 219-224.

Heinrichs, R.W. & Zakzanis, K.K. (1998). Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. *Neuropsychology, 12,* 426-445.

Heishman, S.J. (1999). Behavioral and cognitive effects of smoking: relationship to nicotine addiction. *Nicotine and Tobacco Research, 1,* S143-147.

Hughes, J.R., Hatsukami, D.K., Mitchell, J.E., & Dahlgren, L.A. (1986). Prevalence of smoking among psychiatric outpatients. *American Journal of Psychiatry, 143*, 993-997.

Huxley, N.A., Rendall, M., & Sederer, L. (2000). Psychosocial treatments in schizophrenia: a review of the past 20 years. *Journal of Nervous and Mental Disorders, 188,* 187-201.

Kane, J.M. (2001). Extrapyramidal side effects are unacceptable. *European Neuropsychopharmacology, 11, Suppl. 4,* S397-403.

Kassel, J.D. (1997). Smoking and attention: a review and reformulation of the stimulus-filter hypothesis. *Slinical Psychology Review, 17,* 451-478.

Koch, M. (1998). Sensorimotor gating changes across the estrous cycle in female rats. *Physiology and Behavior, 64,* 625-628.

Kumari, V., Chekley, S.A., & Gray, J. (1996). Effect of cigarette smoking on prepulse inhibition of the acoustic startle reflex in healthy male smokers. *Psychopharmacology, 128,* 54-60.

Kumari, V., Cotter, P.A., Checkley, S.A., & Gray, J.A. (1997). Effect of acute subcutaneous nicotine on prepulse inhibition of the acoustic startle reflex in healthy, male, non-smokers. *Psychopharmacology, 132,* 389-195.

Kyerematen, G.A., Taylor, L.H., de Bethizy, J.D., & Vesell, E.S. (1988). Pharmacokinectics of nicotine and 12 metabolites in the rat. Application of a new radiometric high performance liquid chromatography assay. *Drug Metabolism and Disposition, 16,* 125-129.

Lahti, A.C., Koffel, B., La Porte, D., & Tamminga, C.A. (1995). Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropaychopharmacology, 13,* 9-19.

Leung, A., & Chue, P. (2000). Sex differences in schizophrenia, a review of the literature. *Acta Psychiatrica Scandinavia Supplement, 401,* 3-38.

Lipska, B.K., & Weinberger, D.R. (2000). To model a psychiatric disorder in animals: schizophrenia as a reality test. *Neuropsychophrmaocology, 23,* 223-239.

Love, R.C. (1996). Novel versus conventional antipsychotic drugs. *Pharmacotherapy, 16,* 6-10.

Marcotte, E.R., Pearson, D.M., & Srivastana, L.K. (2001). Animal models of schizophrenia: a critical review. *Journal of Psychiatry and Neuroscience, 26,* 395-410.

Marder, S.R. (2000). Integrating pharmacological and psychosocial treatments for schizophrenia. *Acta Psychiatrica Scandinavia Supplement, 102,* 87-90.

Narrow WE. (1998 – unpublished) One-year prevalence of mental disorders, excluding substance use disorders, in the U.S.: NIMH ECA prospective data. Population estimates based on U.S. Census estimated residential population age 18 and over on July 1, 1998.

Newhouse, P.A., & Hughes, J.R. (1991). The role of nicotine and nicotinic mechanisms in neuropsychiatric disease. *British Journal of Addiction, 86,* 521-526.

Palanza, P. (2001). Animal models of anxiety and depression: how are females different? *Neuroscience and Biobehavioral Reviews, 25,* 219-233.

Perkins, K.A., Grobe, J.E., Epstein, L.H., Caggiula, A. Stiller, R.L. Jacob, R.G. (1994). Chronic and acute tolerance to subjective, behavioral and cardiovascular effects of nicotine in humans. *Journal of Pharmacology and Experimental Therapeutics, 270,* 628-638.

Popke, E.J. (1996, unpublished master's thesis). *Effects of nicotine and nicotinic antagonists on the acoustic startle response and on pre-pulse inhibition in rats.* Bethesda, MD: Uniformed Services University of the Health Sciences.

Popke, E.J., Mayorga, A.J., Fogle, C.M., & Paule, M.G. (2000). Comparison of drug effects on performance iof two timing tasks in rats. *Pharmacology Biochemistry and Behavior, 67,* 377-385.

Provost, S.C., & Woodward, R. (1991). Effects of nicotine gum on repeated administration of the stroop test. *Psychopharmacology, 104,* 536-540.

Rector, N.A., & Beck, A.T. (2001). Cognitive behavioral therapy for schizophrenia: an empirical review. *Journal of Nervous and Mental Disorders, 189,* 278-287.

Regier, D. A., Narrow, W. E., Rae, D. S., Manderscheid, R. W., Locke, B. Z., & Goodwin, F. K. (1993). The de facto US mental and addictive disorders service system. Epidemiologic Catchment Area prospective 1-year prevalence rates of disorders and services. *Archives of General Psychiatry, 50*, 85–94.

Rezvani, A.H., & Levin, E.D. (2001). Cognitive effects of nicotine. *Biological Psychiatry, 49,* 258-267.

Rose, J. (1996). Nicotine addiction and treatment. *Annual Review of Medicine, 47,* 493-507.

Rose, J., & Corrigal, W. (1997). Nicotine self-administration in animals and humans: similarities and differences. *Psychopharmacology, 130*, 28-40.

Sastry, B.V., Chance, M.B., Singh, G., Horn, J.L., & Janson, V.E. (1995). Distribution and retention of nicotine and its metabolite, cotinine, in the rat as a function of time. *Pharmacology, 50,* 128-136.

Scheufele, P., Faraday, M., & Grunberg, N. (2000). Nicotine administration interacts with housing conditions to alter social and non-social behaviors in male and female Long-Evans rats. *Nicotine and Tobacco Research, 2,* 169-178.

Schwartz, P.H., & Wasterlain, C.G. (1991). Cardiac arrest and resuscitation alters the pharmacokinetics of MK-801 in the rat. *Research Communications in Chemical Pathology and Pharmacology, 73,* 183-195.

Smith, G.L. (1996). Smoking, schizophrenia, and boredom. *American Journal of Psychiatry, 153,* 583.

Smith, J.A., Boyer-Millar, C., & Goudie, A.J. (1999). Does MK-801 discrimination constitute an animal model of schizophrenia useful for detecting atypical antipsychotics? *Pharmacology Biochemistry and Behavior* , 64, 429-433.

Spielberger, C.D. (1986). Psychological determinants of smoking behavior. In R.D. Tollison (ed.) *Smoking and society*, Lexington Books: Lexington, MA.

Stahl, S.M. (2000). *Essential psychopharmacology: neuroscientific basis and practical applications (2ⁿᵈ ed.).* Cambridge, UK:Cambridge University Press.

Substance Abuse and Mental Health Services Administration (2000). Summary of Findings from the 1999 National Household Survey on Drug Abuse. Office of Applied Studies, Series: H-12, DHHS Pub. No. (SMA) 00-3466, August 2000.

Swerdlow, N.R., Braff, D.L., Geyer, M.A., & Koob, G.F. (1986). Central dopamine hyperactivity in rats mimics abnormal acoustic startle response in schizophrenics. *Biological Psychiatry, 21,* 23-33.

Swerdlow, N.R., Braff, D.L., Taaid, N., & Geyer, M.A. (1994). Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. *Archives of General Psychiatry, 51,* 139-154.

Swerdlow, N.R., Caine, S.B., Braff, D.L., & Geyer, M.A. (1992). The neural substrates of sensorimotor getting of the startle reflex: a review of recent findings and their implications. *Journal of Psychopharmacology, 6,* 176-190.

Swerdlow, N.R., & Geyer, M.A. (1998). Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophrenia Bulletin, 24,* 285-301.

Tizabi, Y., Mastropaolo, J., Park, C.H., Riggs, R.L., Powell, D., Rosse, R., Deutsch, S.I. (1998). Both nicotine and mecamylamine block dizocilpine-induced explosive jumping behavior in mice: psychiatric implications. *Psychopharmacology, 140,* 202-205.

Treit, D. (1985). Animal models for the study of anti-anxiety agents: a review. *Neuroscience and Biobehavioral Reviews, 9,* 203-222.

United States Department of Health and Human Services. (1988). The Health Consequences of Smoking: Nicotine Addiction: A Report of the Surgeon General. Office on Smoking and Health.

United States Department of Health and Human Services. (1994). Preventing Tobacco Use Among Young People: A report of the Surgeon General. Office on Smoking and Health.

United States Department of Health and Human Services. (1999). Mental Health: A Report of the Surgeon General.

Varty, G.B., & Higgins, G.A. (1995). Examiniation of drug-induced and isolation-induced disruptions of pre-pulse inhibition as models to screen antipsychotic drugs. *Psychopharmacology, 122,* 15-26.

Varty, G.B., Bakshi, V.P., & Geyer, M.A. (1999). M100907, a serotonin 5-HT2A receptor antagonist and putative antipsychotic, blocks dizocilpine-induced pre-pulse inhibition deficits in Sprague-Dawley and Wistar rats. *Neuropsychopharmacology, 20,* 311-321.

Weiss, I.C., & Feldon, J. (2001). Environmental animal models for sensorimotor gating deficiencies in schizophrenia: a review. *Psychopharmacology, 156,* 305-326.

Wesnes, K. & Warburton, D.M. (1983). Effects of smoking on rapid information processing performance. *Neuropsychobiology, 9,* 223-229.

Wesnes, K., & Warburton, D.M. (1984). The effects of cigarettes of varying yield on rapid information processing performance. *Psychopharmacology, 82,* 338-342.

Willetts, J., Balster, R.L., & Leander, J.D. (1990). The behavioral pharmacology of NMDA receptor antagonists. *Trends in Pharmacological Science, 11,* 423-428.

Winders, S.E., & Grunberg, N.E. (1990). Effects of nicotine on bodyweight, food consumption, and body composition in male rats. *Life Sciences, 46,* 1523-1530.

Winders, S.E., Grunberg, N.E., Benowitz, N.L., & Alvarez, A.P. (1998). Effects of stress on circulating nicotine and cotinine levels an invitro metabolism in the rat. *Psychopharmacology, 137,* 383-390.

Ziedonis, D.M., Kosten, T.R., Glazer, W.M., & Frances, R.J. (1994). Nicotine dependence and schizophrenia. *Hospital community Psychiatry, 45*, 204-206.

Zubin, J., & Spring, B. (1977). Vulnerability—a new view of schizophrenia. *Journal of Abnormal Psychology, 86*, 103–126.